Agilent 1200 Series Fluorescence Detector

G1321A

Service Manual

Agilent Technologies

Notices

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Manual Structure

The **Service Manual G1321-90110** (English) contains the complete information about the Agilent 1200 Series Fluorescence Detector. It is available as Adobe Reader file (PDF) only.

The **User Manual G1321-90010** (English) and its localized versions contain a subset of the Service Manual and is shipped with the detector in printed matter.

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In This Guide...

1 Introduction to the Fluorescence Detector

This chapter gives an introduction to the detector, instrument overview and internal connectors.

2 Site Requirements and Specifications

This chapter gives information on environmental requirements, physical and performance specifications.

3 Installing the Detector

This chapter describes the installation of the detector.

4 First Steps with the Detector

This chapter guides you how to start the work with the detector.

5 How to optimize the Detector

This chapter provides information on how to optimize the detector.

6 Troubleshooting and Test Functions

This chapter gives an overview about the troubleshooting and diagnostic features and the different user interfaces.

7 Error Information

This chapter describes the meaning of detector error messages, and provides information on probable causes and suggested actions how to recover from error conditions.

8 Test Functions

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This chapter gives an introduction to the detector, instrument overview and internal connectors.

Introduction to the Detector

The detector is designed for highest optical performance, GLP compliance and easy maintenance. It includes the following features:

- flash lamp for highest intensity and lowest detection limit
- multi-wavelength mode for on-line spectra
- spectra acquisition and simultaneous multi-signal detection
- optional cuvette is available and can be used for off-line measurements
- easy front access to flow cell for fast replacement and
- built-in wavelength accuracy verification.

For specifications, see “Performance Specifications” on page 43

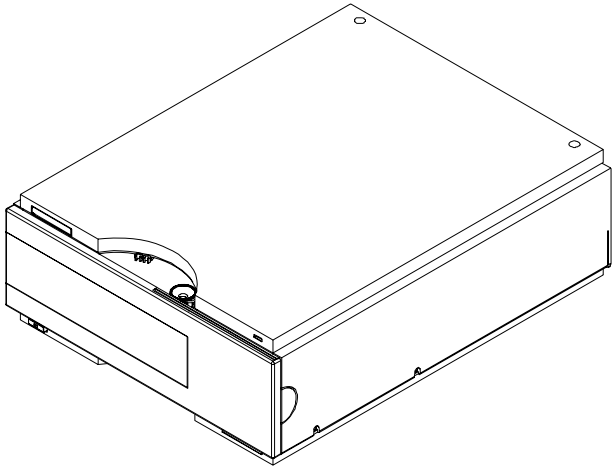


Figure 1 The Agilent 1200 Series Fluorescence Detector.

How the Detector Operates

Luminescence Detection

Luminescence, the emission of light, occurs when molecules change from an excited state to their ground state. Molecules can be excited by different forms of energy, each with its own excitation process. For example, when the excitation energy is light, the process is called *photoluminescence*.

In basic cases, the emission of light is the reverse of absorption, see Figure 2. With sodium vapor, for example, the absorption and emission spectra are a single line at the same wavelength. The absorption and emission spectra of organic molecules in solution produce bands instead of lines.

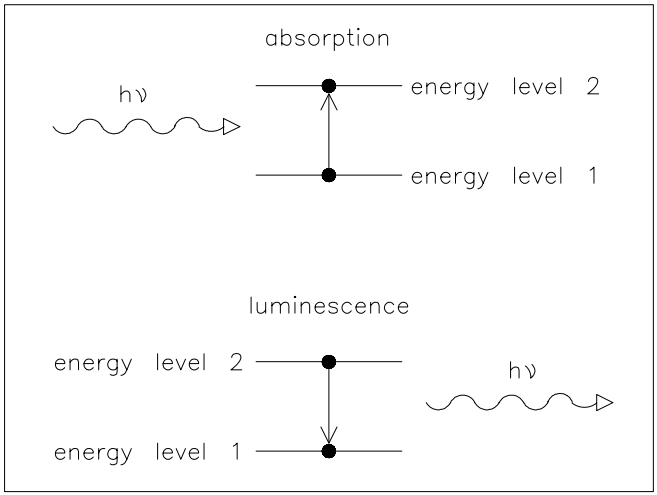


Figure 2 Absorption of Light Versus Emission of Light

When a more complex molecule transforms from its ground energy state into an excited state, the absorbed energy is distributed into various vibrational and rotational sub-levels. When this, same molecule returns to the ground state, this vibrational and rotational energy is first lost by relaxation without any radiation. Then the molecule transforms from this energy level to one of the vibrational and rotational sub-levels of its ground state, emitting light, see Figure 3. The characteristic maxima of absorption for a substance is its λ_{EX}, and for emission its λ_{EM}.

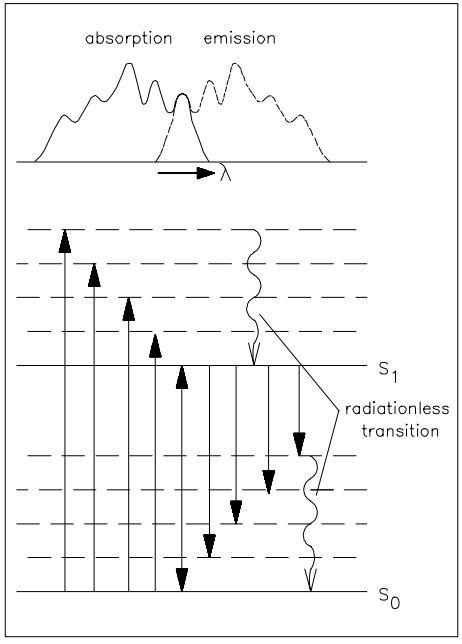


Figure 3 Relationship of Excitation and Emission Wavelengths

Photoluminescence is the collective name for two phenomena, *fluorescence* and *phosphorescence*, which differ from each other in one characteristic way--the delay of emission after excitation. If a molecule emits light 10^{-9} to 10^{-5} seconds after it was illuminated then the process was fluorescence. If a molecule emits light longer than 10^{-3} seconds after illumination then the process was phosphorescence.

Phosphorescence is a longer process because one of the electrons involved in the excitation changes its spin, during a collision with a molecule of solvent, for example. The excited molecule is now in a so-called triplet state, T, see Figure 4.

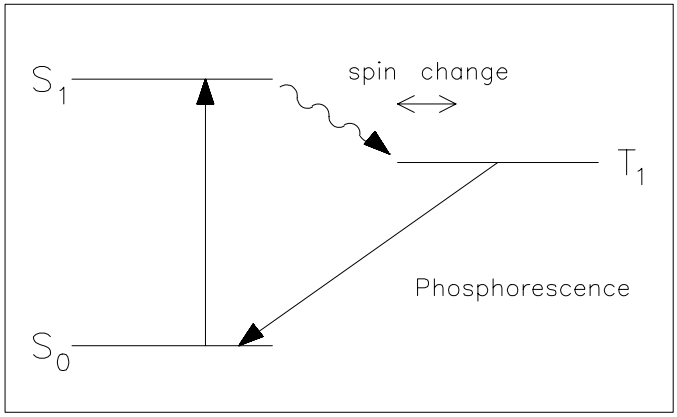


Figure 4 Phosphorescence Energy Transitions

The molecule must change its spin back again before it can return to its ground state. Since the chance of colliding with another molecule with the necessary spin for change is slight, the molecule remains in its triplet state for some time. During the second spin change the molecule loses more energy by relaxing without radiation. The light which is emitted during phosphorescence therefore has less energy and is at a longer wavelength than fluorescence.

Formula: $E = h \times \lambda^{-1}$

In this equation:

E is energy
h is Planck's constant
λ is the wavelength

Raman Effect

The Raman effect arises when the incident light excites molecules in the sample which subsequently scatter the light. While most of this scattered light is at the same wavelength as the incident light, some is scattered at a different wavelength. This inelastically scattered light is called Raman scatter. It results from the molecule changing it's molecular motions.

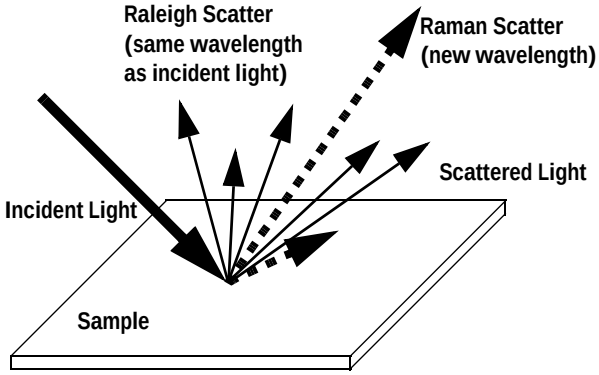


Figure 5 Raman

The energy difference between the incident light (E_i) and the Raman scattered light (E_s) is equal to the energy involved in changing the molecule's vibrational state (i.e. getting the molecule to vibrate, E_v). This energy difference is called the Raman shift.

$$E_v = E_i - E_s$$

Several different Raman shifted signals will often be observed; each being associated with different vibrational or rotational motions of molecules in the sample. The particular molecule and its environment will determine what Raman signals will be observed (if any).

A plot of Raman intensity versus Raman shift is a Raman spectrum.

Optical Unit

All the elements of the optical system, shown in Figure 6 on page 21, including Xenon flash lamp, excitation condenser, excitation slit, mirror, excitation grating, flow cell, emission condenser, cut-off filter, emission slit, emission grating and photo-multiplier tube are housed in the metal casting inside the detector compartment. The fluorescence detector has grating/grating optics, enabling the selection of both excitation and emission wavelengths. The flow cell can be accessed from the front of the fluorescence detector.

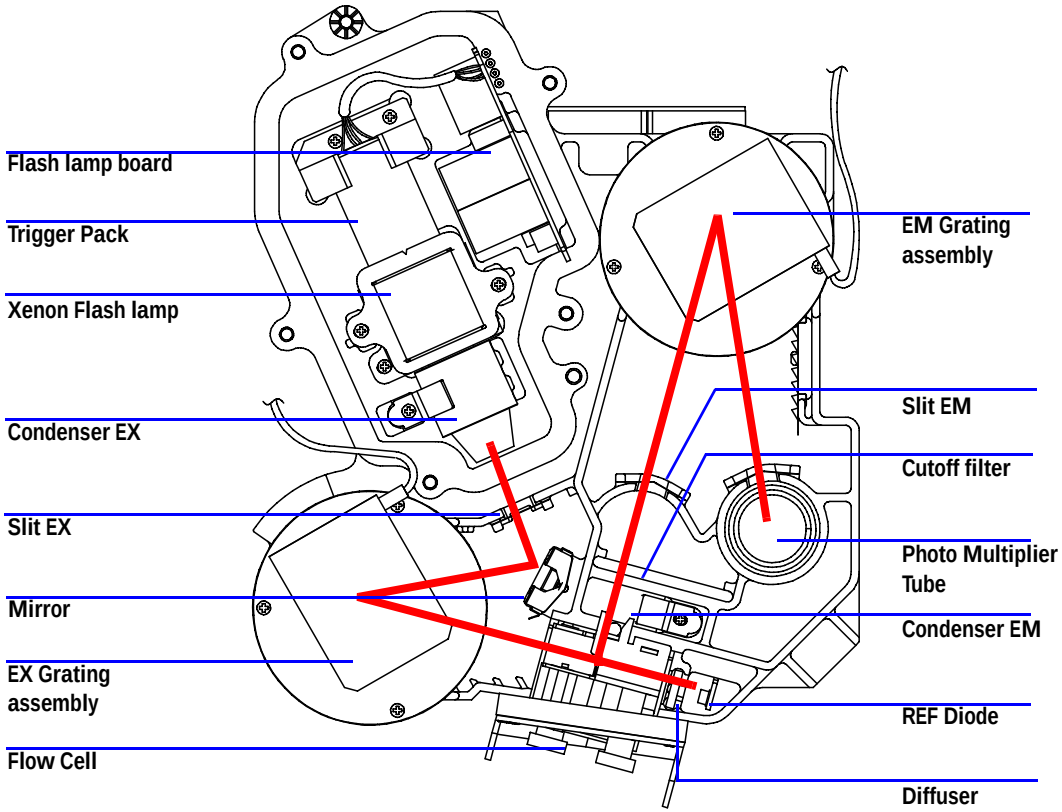


Figure 6 Optical Unit

The radiation source is a xenon flash-lamp. The 3 µs flash produces a continuous spectrum of light from 200 nm to 900 nm. The light output distribution can be expressed as a percentage in 100 nm intervals, see Figure 7. The lamp can be used for some 1000 hours depending on the sensitivity requirements. You can economize during automatic operation using keyboard setpoints, so the lamp flashes during your analysis only. The lamp can be used until it no longer ignites, but the noise level may increase with usage.

UV degradation, especially below 250 nm is significantly higher compared to Visible wavelength range. Generally the "LAMP ON during run" - setting or using "economy mode" will increase lamp life by a magnitude.

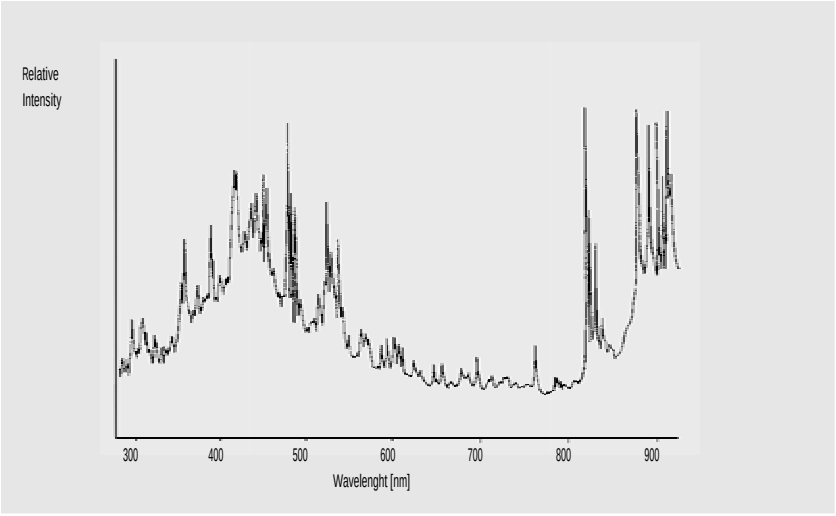


Figure 7 Lamp Energy Distribution (vendor data)

The radiation emitted by the lamp is dispersed and reflected by the excitation monochromator grating onto the cell entrance slit.

The holographic concave grating is the main part of the monochromator, dispersing and reflecting the incident light. The surface contains many minute grooves, 1200 of them per millimeter. The grating carries a blaze to show improved performance in the visible range.

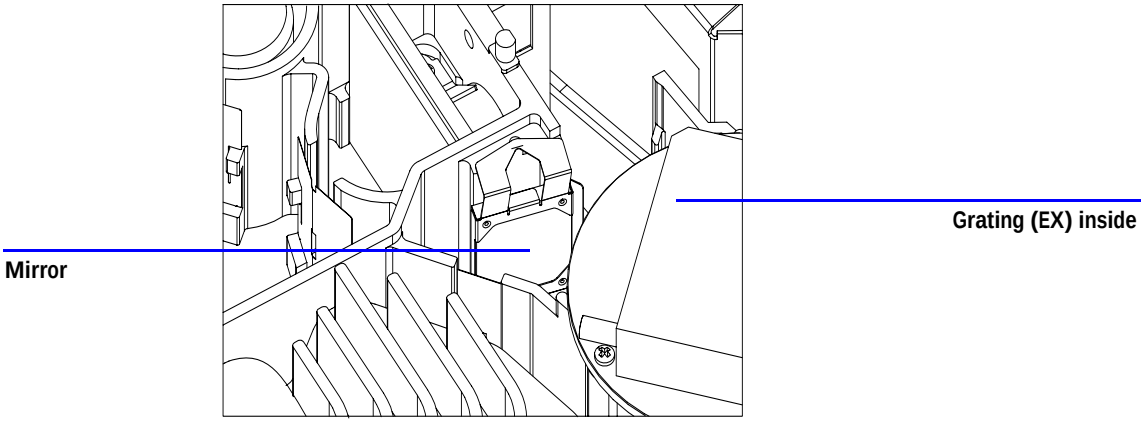


Figure 8 Mirror Assembly

The geometry of the grooves is optimized to reflect almost all of the incident light, in the 1st order and disperse it with about 70% efficiency in the ultra-violet range. Most of the remaining 30% of the light is reflected at zero order, with no dispersion. Figure 9 illustrates the light path at the surface of the grating.

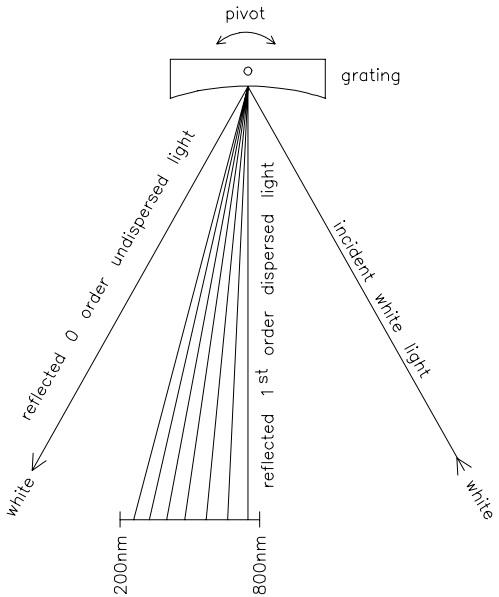


Figure 9 Dispersion of Light by a Grating

The grating is turned using a 3-phase brushless DC motor, the position of the grating determining the wavelength or wavelength range of the light falling onto the flow cell. The grating can be programmed to change its position and therefore the wavelength during a run.

For spectra acquisition and multi-wavelength detection, the grating rotates at 4000 rpm.

The excitation and emission gratings are similar in design, but have different blaze wavelengths. The excitation grating reflects most 1st order light in the ultra-violet range around 250 nm, whereas the emission grating reflects better in the visible range around 400 nm.

The flow cell is a solid quartz body with a maximum back pressure of 20 bar. Excessive back pressure will result in destruction of the cell. Operating the detector close to waste with low back pressure is recommended. A slit is integrated to the quartz body.

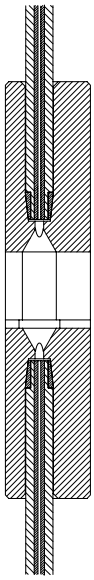


Figure 10 Cross-Section of Flow Cell

The luminescence from the sample in the flow cell is collected at right angles to the incident light by a second lens, and passes through a second slit. Before the luminescence reaches the emission monochromator, a cut-off filter removes light below a certain wavelength, to reduce noise from 1st order scatter and 2nd order stray light, see Figure 9 on page 24.

The selected wavelength of light is reflected onto the slit in the wall of the photo-multiplier compartment of the optical unit. The bandwidth of the emitted light is 20 nm.

On the photocathode, Figure 11, incident photons generate electrons. These electrons are accelerated by an electrical field between several arc-shaped dynodes. Depending on the voltage difference between any pair of dynodes, an incident electron may spark-off further electrons which accelerate onto the next dynode. An avalanche effect results: finally so many electrons are generated that a current can be measured. The amplification is a function of the voltage at the dynodes and is microprocessor controlled. You can set the amplification using the PMTGAIN function.

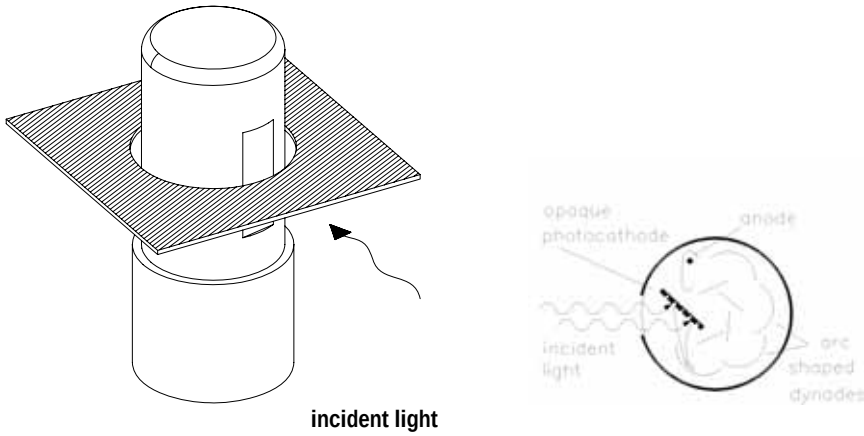


Figure 11 Photo-multiplier Tube

This type of so-called side-on photo-multiplier is compact ensuring fast response, conserving the advantages of the short optical path shown in Figure 6 on page 21.

PMTs are designed for specific wavelength ranges. The standard PMT offers optimum sensitivity from 200 to 600 nm. In the higher wavelength range a red-sensitive PMT can improve performance. For additional PMT types refer to"Spare Parts" on page 262.

Reference System

A reference diode, located behind the flow cell, measures the excitation (EX) light transmitted by the flow cell and corrects flash lamp fluctuations and long-term intensity drift. Because of a non-linear output of the diode (depending on the EX-wavelength), the measured data are normalized.

A diffuser is located in front of the reference diode (see Figure 6 on page 21). This diffuser is made of quartz, reduces light and allows integral measurement of the light.

Analytical Information From Primary Data

We now know how the primary data from your sample is acquired in the optical unit. But how can the data be used as information in analytical chemistry? Depending on the chemistry of your application, the luminescence measured by the fluorescence detector will have different characteristics. You must decide, using your knowledge of the sample, what mode of detection you will use.

Fluorescence Detection

When the lamp flashes, the fluorescing compounds in your sample will luminesce almost simultaneously, see Figure 12. The luminescence is short-lived, therefore the fluorescence detector need only measure over a short period of time after the lamp has flashed.

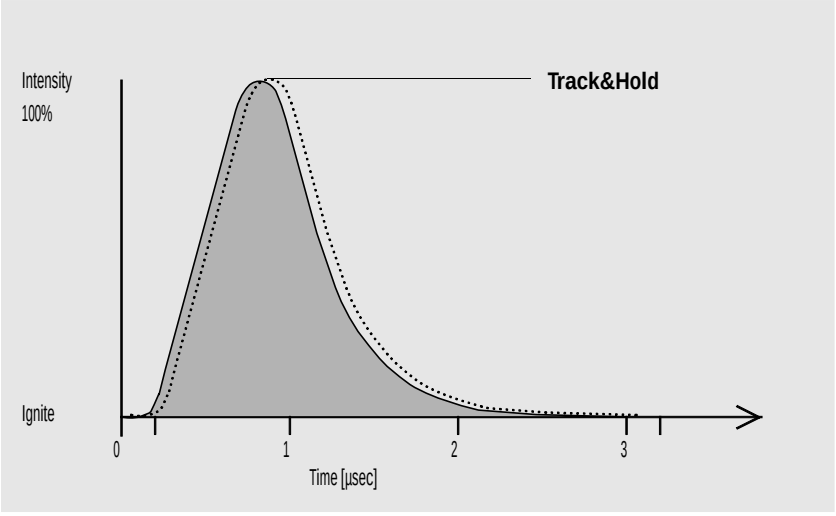


Figure 12 Measurement of Fluorescence

Phosphorescence Detection

An appropriate parameter set will be specified as soon as you chose the phosphorescence detection mode (special setpoints under FLD parameter settings).

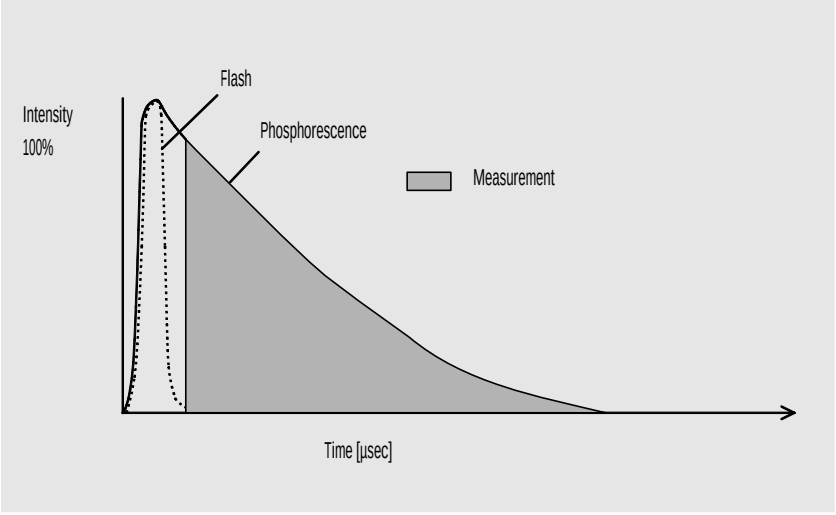


Figure 13 Measurement of Phosphorescence

Processing of Raw Data

If the lamp flashes at single wavelength and high-power, then the fluorescence data rate is 296 Hz. That means that your sample is illuminated 296 times per second, and any luminescence generated by the components eluted from the column is measured 296 times per second.

If the “economy” or multi-wavelength mode is set, then the flash frequency is 74 Hz.

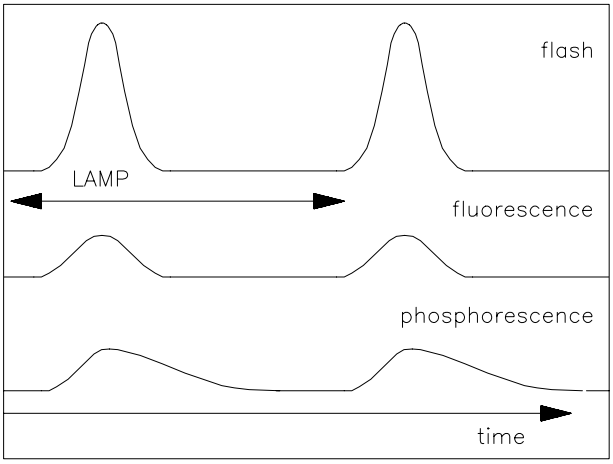


Figure 14 LAMP: Frequency of Flash, Fluorescence, and Phosphorescence

You can improve the signal-to-noise characteristics by disabling the “economy” mode.

NOTE Disabling the “economy” mode will shorten the lifetime of the lamp significantly. Consider lifetime saving by switching off the lamp after the run is completed.

The data resolution is 20 bit at a response time of 4 seconds (default, which is equivalent to a time constant of 1.8 seconds and appropriate for standard chromatographical conditions). Weak signals may cause errors in

quantification because of insufficient resolution. Check your proposed PMTGAIN. If it is significantly distant from your setting, change your method or check the purity of your solvent.

You can amplify the signal using PMTGAIN. Depending on the PMTGAIN you have set, a multiple of electrons is generated for every photon falling on the photomultiplier. You can quantify large and small peaks in the same chromatogram by adding PMTGAIN changes during the run into a timetable.

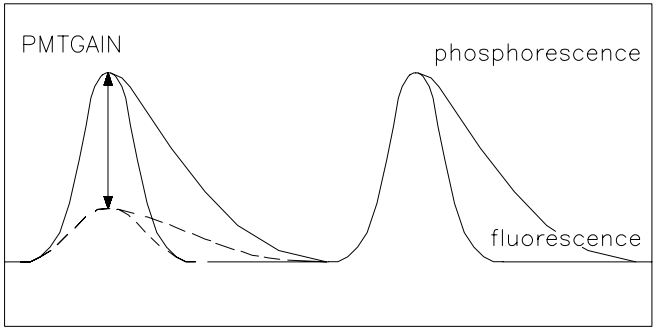


Figure 15 PMTGAIN: Amplification of Signal

Check proposed PMTGAIN. Deviations of more than 2 PMT gains should be corrected in the method.

Each PMTGAIN step is increased approximately by a factor of 2 (range 0 - 18). To optimize your amplification for the peak with the highest emission, raise the PMTGAIN setting until the best signal-to-noise is achieved.

After the photons are converted and multiplied into an electronic signal, the signal (at present analog) is tracked and held beyond the photo-multiplier. After being held, the signal is converted by an A-to-D converter to give one raw data point (digital). Eleven of these data points are bunched together as the first step of data processing. Bunching improves your signal-to-noise ratio.

The bunched data, shown as larger black dots in Figure 16, is then filtered using a boxcar filter. The data is smoothed, without being reduced, by taking the mean of a number of points. The mean of the same points minus the first plus the next, and so on, is calculated so that there are the same number of

bunched and filtered points as the original bunched points. You can define the length of the boxcar element using the RESPONSETIME function: the longer the RESPONSETIME, the greater the number of data points averaged. A four-fold increase in RESPONSETIME (for example, 1 sec to 4 sec) doubles the signal-to-noise ratio.

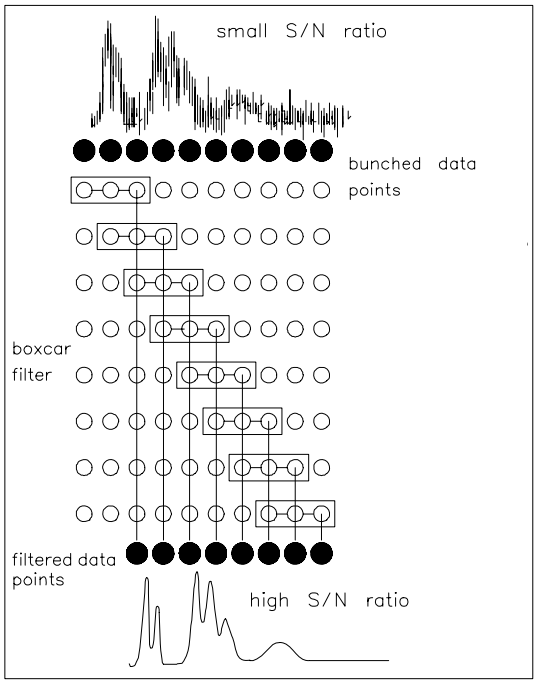


Figure 16 RESPONSETIME: Signal-to-Noise Ratio

Electrical Connections

- The GPIB connector is used to connect the detector with a computer. The address and control switch module next to the GPIB connector determines the GPIB address of your detector. The switches are preset to a default address (see "GPIB Default Addresses" on page 321) which is recognized once the power is switched on.
- The CAN bus is a serial bus with high speed data transfer. The two connectors for the CAN bus are used for internal Agilent 1200 Series module data transfer and synchronization.
- Two independent analog outputs provide signals for integrators or data handling systems.
- The interface board slot is used for external contacts and BCD bottle number output or LAN connections.
- The REMOTE connector may be used in combination with other analytical instruments from Agilent Technologies if you want to use features such as start, stop, common shut down, prepare, and so forth.
- With the appropriate software, the RS-232C connector may be used to control the detector from a computer through a RS-232C connection. This connector is activated and can be configured with the configuration switch next to the GPIB connector (see "Communication Settings for RS-232C Communication" on page 322). See your software documentation for further information.

  Together with a Control Module G1323B the RS-232C may be used to print screens to a connected printer.
- The power input socket accepts a line voltage of 100 – 240 V AC ± 10 % with a line frequency of 50 or 60 Hz. Maximum power consumption is 220 VA. There is no voltage selector on your detector because the power supply has a wide-ranging capability. There are no externally accessible fuses, because automatic electronic fuses are implemented in the power supply. The security lever at the power input socket prevents removal of the detector cover when line power is still connected.

WARNING **Never use cables other than the ones supplied by Agilent Technologies to ensure proper functionality and compliance with safety or EMC regulations.**

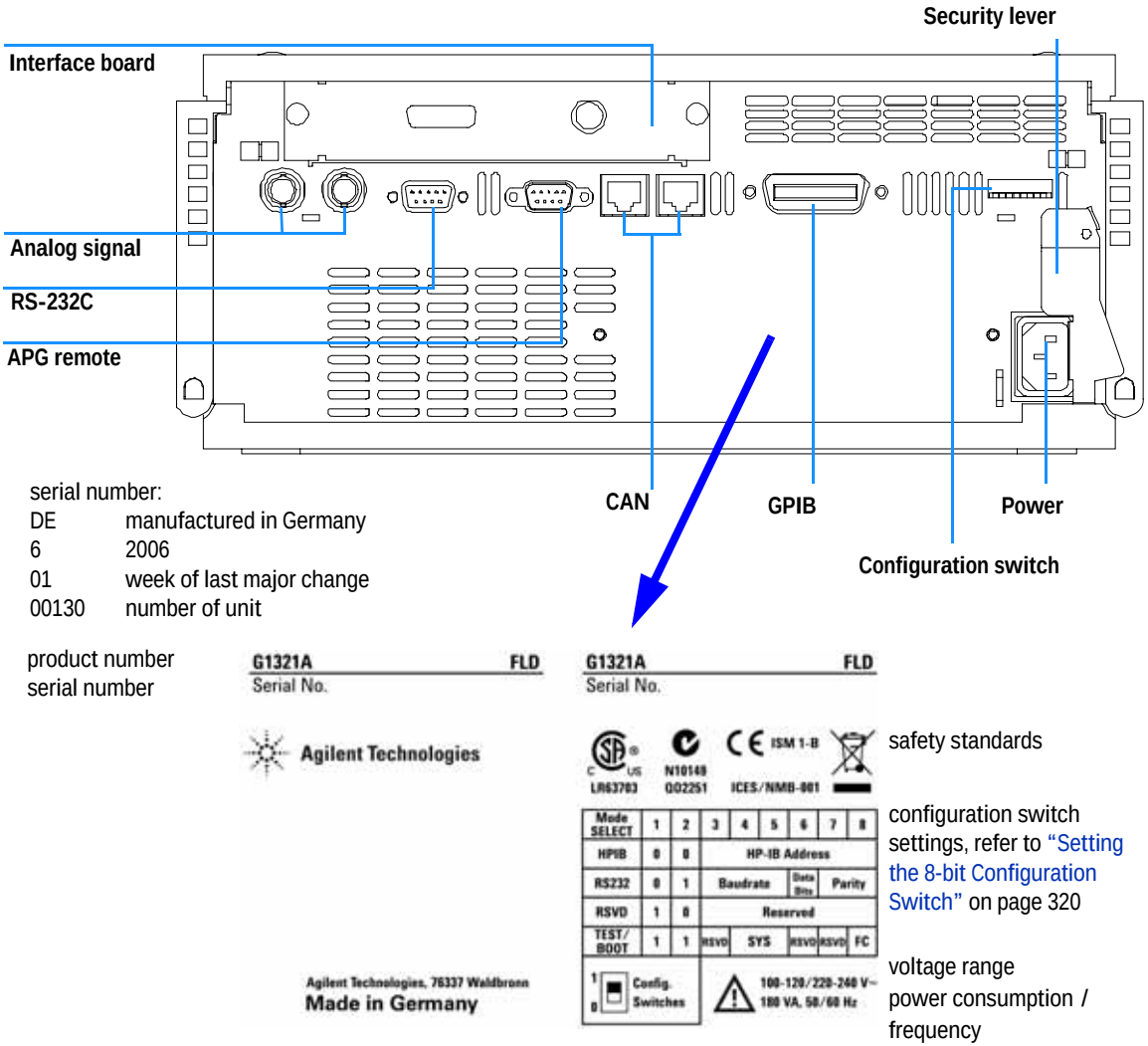


Figure 17 Electrical Connections

Instrument Layout

The industrial design of the detector incorporates several innovative features. It uses Agilent's E-PAC concept for the packaging of electronics and mechanical assemblies. This concept is based upon the use of expanded polypropylene (EPP) layers of foam plastic spacers in which the mechanical and electronic boards components of the detector are placed. This pack is then housed in a metal inner cabinet which is enclosed by a plastic external cabinet. The advantages of this packaging technology are:

- virtual elimination of fixing screws, bolts or ties, reducing the number of components and increasing the speed of assembly/disassembly,
- the plastic layers have air channels molded into them so that cooling air can be guided exactly to the required locations,
- the plastic layers help cushion the electronic and mechanical parts from physical shock, and
- the metal inner cabinet shields the internal electronics from electromagnetic interference and also helps to reduce or eliminate radio frequency emissions from the instrument itself.

Early Maintenance Feedback (EMF)

Maintenance requires the exchange of components which are subject to wear or stress. Ideally, the frequency at which components are exchanged should be based on the intensity of usage of the detector and the analytical conditions, and not on a predefined time interval. The early maintenance feedback (EMF) feature monitors the usage of specific components in the instrument, and provides feedback when the user-selectable limits have been exceeded. The visual feedback in the user interface provides an indication that maintenance procedures should be scheduled.

EMF Counters

The detector provides three EMF counters for the lamp. The counters increment with lamp use, and can be assigned a maximum limit which provides visual feedback in the user interface when the limit is exceeded. The counters can be reset to zero after the lamp is exchanged. The detector provides the following EMF counters:

- number of flashes (low power mode, multiples of 1000 flashes)
- number of flashes (high power mode, multiples of 1000 flashes)
- Flash Lamp Life-Time (value of 0 – 100% as expected lifetime factor calculated from combined expected lifetime low power and high power flashes).

Figure 18 on page 37 shows the lamp life based on number of flashes vs. the input energy. The lamp flash frequency/energy can be changed into the following modes:

Table 1 Flash Lamp Modes

Position	296 Hz (Standard), 560 V	63 mJoule (18.8 W)
	74 Hz (Economy), 560 V	63 mJoule (4.7 W)
Rotation (Multi Ex/Em)	74 Hz (Standard), 950 V	180 mJoule (13.3 W)
	74 Hz (Economy), 560 V	63 mJoule (4.7 W)

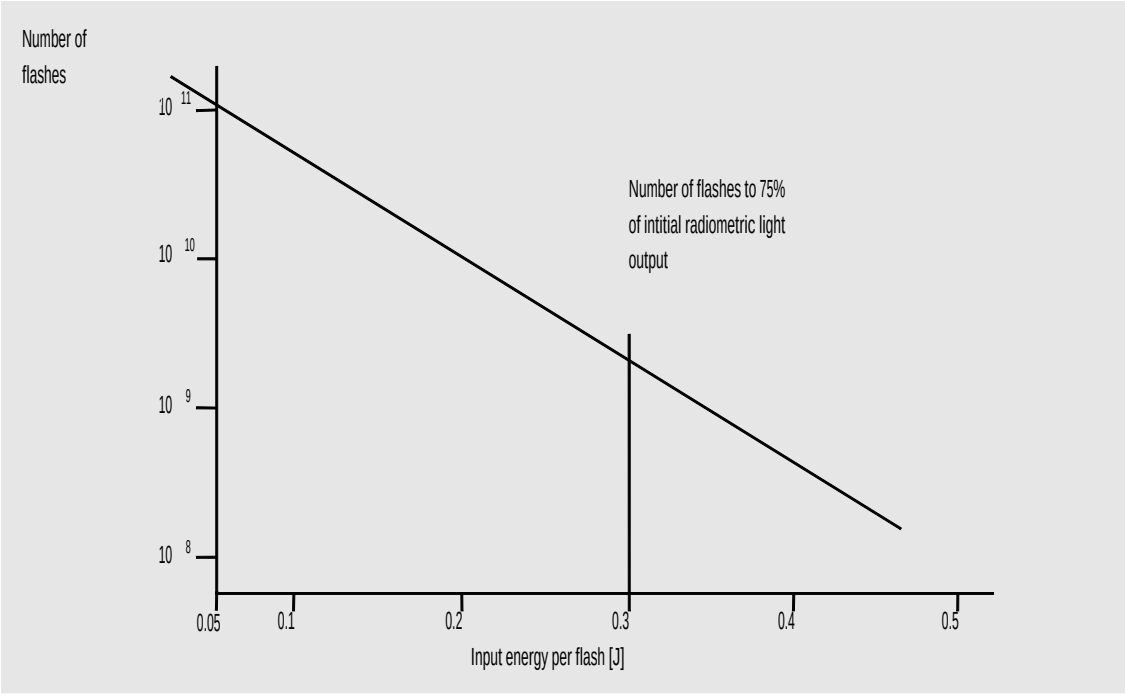


Figure 18 Lamp life

Using the EMF Counters

The user-selectable EMF limits for the EMF counters enable the early maintenance feedback to be adapted to specific user requirements. The useful lamp burn time is dependent on the requirements for the analysis (high or low sensitivity analysis, wavelength etc.), therefore, the definition of the maximum limits need to be determined based on the specific operating conditions of the instrument.

Setting the EMF Limits

The setting of the EMF limits must be optimized over one or two maintenance cycles. Initially, no EMF limit should be set. When instrument performance indicates maintenance is necessary, take note of the values displayed by lamp counters. Enter these values (or values slightly less than the displayed values) as EMF limits, and then reset the EMF counters to zero. The next time the EMF counters exceed the new EMF limits, the EMF flag will be displayed, providing a reminder that maintenance needs to be scheduled.

2
Site Requirements and Specifications

Site Requirements 40
Physical Specifications 42
Performance Specifications 43

This chapter gives information on environmental requirements, physical and performance specifications.

Site Requirements

A suitable environment is important to ensure optimal performance of the detector.

Power Consideration

The detector power supply has wide ranging capabilities and accepts any line voltage in the range mentioned in Table 2 on page 42. Consequently, there is no voltage selector in the rear of the detector. There are also no externally accessible fuses, because automatic electronic fuses are implemented in the power supply.

WARNING **To disconnect the detector from line, unplug the power cord. The power supply still uses some power, even if the power switch on the front panel is turned off.**

WARNING **Shock hazard or damage of your instrumentation can result, if the devices are connected to a line voltage higher than specified.**

CAUTION Make sure to have easy access to the power cable of the instrument, in order to disconnect the instrument from line.

Power Cords

Different power cords are offered as options with the detector. The female end of all power cords is identical. It plugs into the power-input socket at the rear of the detector. The male end of each power cord is different and designed to match the wall socket of a particular country or region.

WARNING **Never operate your instrumentation from a power outlet that has no ground connection. Never use a power cord other than the Agilent Technologies power cord designed for your region.**

WARNING **Never use cables other than the ones supplied by Agilent Technologies to ensure proper functionality and compliance with safety or EMC regulations.**

Bench Space

The detector dimensions and weight (see Table 2 on page 42) allows you to place the detector on almost any desk or laboratory bench. It needs an additional 2.5 cm (1.0 inches) of space on either side and approximately 8 cm (3.1 inches) in the rear for air circulation and electric connections.

If the bench should carry a Agilent 1200 Series system, make sure that the bench is designed to bear the weight of all modules.

The detector should be operated in a horizontal position.

Environment

Your detector will work within the specifications at ambient temperatures and relative humidity described in Table 2 on page 42.

ASTM drift tests require a temperature change below 2 °C/hour (3.6 °F/hour) over one hour period. Our published drift specification (refer also to "Performance Specifications" on page 43) is based on these conditions. Larger ambient temperature changes will result in larger drift.

Better drift performance depends on better control of the temperature fluctuations. To realize the highest performance, minimize the frequency and the amplitude of the temperature changes to below 1 °C/hour (1.8 °F/hour). Turbulences around one minute or less can be ignored.

CAUTION Do not store, ship or use your detector under conditions where temperature fluctuations could cause condensation within the detector. Condensation will damage the system electronics. If your detector was shipped in cold weather, leave it in its box and allow it to warm up slowly to room temperature to avoid condensation.

Physical Specifications

Table 2 Physical Specifications

Type	Specification	Comments
Weight	11.5 kg (26 lbs)	
Dimensions (width × depth × height)	345 × 435 × 140 mm (13.5 × 17 × 5.5 inches)	
Line voltage	100 – 240 VAC, ± 10 %	Wide-ranging capability
Line frequency	50 or 60 Hz ± 5 %	
Power consumption	180 VA / 70 W / 239 BTU	Maximum
Ambient operating temperature	0 – 40 °C (32 – 104 °F)	
Ambient non-operating temperature	-40 – 70 °C (-4 – 158 °F)	
Humidity	< 95%, at 25 – 40 °C (77 – 104 °F)	Non-condensing
Operating altitude	Up to 2000 m (6500 ft)	
Non-operating altitude	Up to 4600 m (14950 ft)	For storing the detector
Safety standards: IEC, CSA, UL, EN	Installation category II, pollution degree 2. For indoor use only.	

Performance Specifications

Table 3 Performance Specifications Agilent 1200 Series Fluorescence Detector

Type	Specification	Comments
Detection type	Multi-signal fluorescence detector with rapid on-line scanning capabilities and spectral data analysis	
Performance Specifications	10 fg Anthracene, Ex=250 nm, Em=400 nm*	see note below this table
	RAMAN single wavelength (H_2O) > 500 with Ex=350 nm, Em=397 nm, dark value 450 nm, standard flow cell time constant=4 seconds (8 seconds responsetime)	see "Raman ASTM Signal-to-Noise Test" on page 144
	RAMAN dual wavelength (H_2O) > 300 with Ex=350 nm, Em=397 nm, dark value 450 nm, standard flow cell time constant=4 seconds (8 seconds responsetime)	see "Raman ASTM Signal-to-Noise Test" on page 144
Light source	Xenon Flash Lamp, normal mode 20 W, economy mode 5 W	
Pulse frequency	296 Hz for single signal mode 74 Hz for spectral mode	
Excitation Monochromator	Range:200 nm - 700 nm and zero-order Bandwidth:20 nm (fixed) Monochromator:concave holographic grating, F/1.6, blaze: 300 nm	
Emission Monochromator	Range:280 nm - 900 nm and zero-order Bandwidth:20 nm (fixed) Monochromator:concave holographic grating, F/1.6, blaze: 400 nm	
Reference System:	in-line excitation measurement	

Table 3 Performance Specifications Agilent 1200 Series Fluorescence Detector

Type	Specification	Comments
Timetable programing:	up to 4 signal wavelengths, response time, PMT Gain, baseline behavior (append, free, zero), spectral parameters	
Spectrum acquisition:	Excitation or Emission spectra Scan speed: 28 ms per datapoint (e.g. 0.6 s/spectrum 200-400 nm, 10 nm step) Step size: 1-20 nm Spectra storage: All	
Wavelength characteristic	Repeatability+/- 0.2 nm Accuracy+/- 3 nm setting	
Flow cells	Standard: 8 µl volume and 20 bar (2 MPa) pressure maximum, quartz Optional: Fluorescence cuvette for offline spectroscopic measurements with 1 ml syringe, 8 µl volume, quartz	
Control and data evaluation	Agilent ChemStation for LC, Agilent Instant Pilot G4208A or Agilent Control Module G1323B with limited spectral data analysis and printing of spectra	
Analog outputs	Recorder/integrator: 100 mV or 1 V, output range >10^2 luminescence units, two outputs	
Communications	Controller-area network (CAN), GPIB, RS-232C, LAN, APG Remote: ready, start, stop and shut-down signals	

Table 3 Performance Specifications Agilent 1200 Series Fluorescence Detector

Type	Specification	Comments
Safety and maintenance	Extensive diagnostics, error detection and display (through Instant Pilot G4208A, Control Module G1323B and ChemStation), leak detection, safe leak handling, leak output signal for shutdown of pumping system. Low voltages in major maintenance areas.	
GLP features	Early maintenance feedback (EMF) for continuous tracking of instrument usage in terms of lamp burn time with user-settable limits and feedback messages. Electronic records of maintenance and errors. Verification of wavelength accuracy, using the Raman band of water.	
Housing	All materials recyclable.	
Environment:	0 to 40 °C constant temperature at <95% humidity (non-condensing)	
Dimensions:	140 mm x 345 mm x 435 mm (5.5 x 13.5 x 17 inches) (height x width x depth)	
Weight:	11.5 kg (25.5 lbs)	

NOTE Reference conditions: standard cell 8 µl, response time 4 s, flow 0.4 ml/min LC-grade Methanol, 2.1 x 100 mm ODS column.

3
Installing the Detector

Unpacking the Detector 48
Optimizing the Stack Configuration 51
Installing the Detector 54
Flow Connections to the Detector 57

This chapter describes the installation of the detector.

Unpacking the Detector

Damaged Packaging

If the delivery packaging shows signs of external damage, please call your Agilent Technologies sales and service office immediately. Inform your service representative that the detector may have been damaged during shipment.

CAUTION If there are signs of damage, please do not attempt to install the detector.

Delivery Checklist

Ensure all parts and materials have been delivered with the detector. The delivery checklist is shown below. Please report missing or damaged parts to your local Agilent Technologies sales and service office.

Table 4 Detector Checklist

Description	Quantity
Detector	1
Power cable	1
CAN cable	1
Flow cell	1 (built-in)
Optional flow cell/cuvette	as ordered
User Manual	1
Accessory kit (see Table 5 on page 49)	1

Detector Accessory Kit Contents

Table 5 Accessory Kit Contents (Part Number G1321-68705)

Description	Part Number	Quantity
Teflon Tubing flexible i.d. 0.8 mm (flow cell to waste), re-order 5 m	5062-2462	2 m
Corrugated tubing (to waste), re-order 5 m	5062-2463	1.2 m
Fitting male PEEK	0100-1516	2
Capillary column-detector, one side preinstalled 380 mm lg, 0.17 mm i.d. includes:	G1315-87311	1
Ferrule front SST	0100-0043	1
Ferrule back SST	0100-0044	1
Fitting SST	79814-22406	1
Hex key set 1 – 5 mm	8710-0641	1
Screwdriver hexagonal 4 mm, 100 mm long	5965-0027	1
Screwdriver hexagonal 2.5 mm, 100 mm long	5965-0028	1
Needle-Syringe	9301-0407	
Glass-Syringe	9301-1446	
Calibration Sample, Glycogen	5063-6597	
Sample filter, diameter=3 mm, pore size 0.45 µm	5061-3367 (pack of 100)	5
Wrench open end 1/4 – 5/16 inch	8710-0510	1

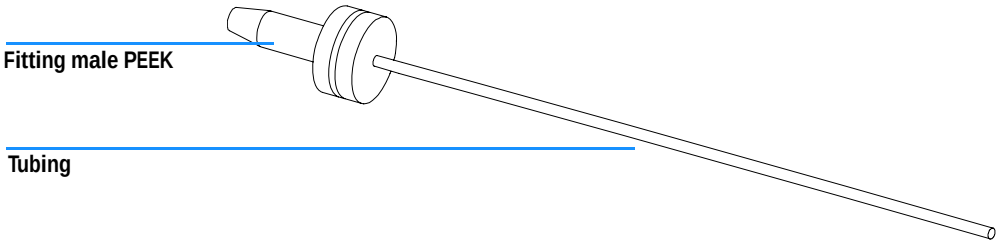


Figure 19 Waste Tubing Parts

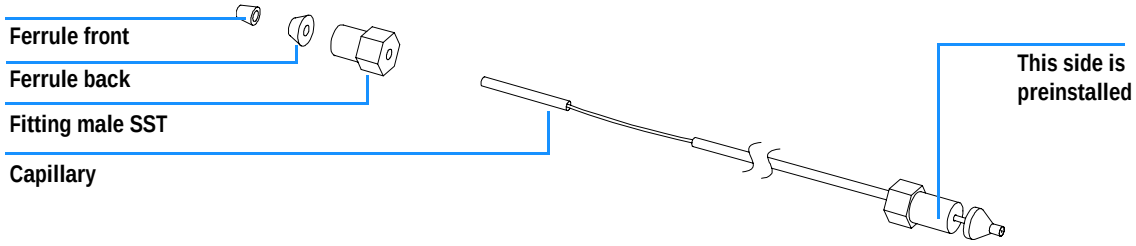


Figure 20 Inlet Capillary (Column-Detector) Parts

Optimizing the Stack Configuration

If your detector is part of a complete Agilent 1200 Series system, you can ensure optimum performance by installing the following configuration. This configuration optimizes the system flow path, ensuring minimum delay volume.

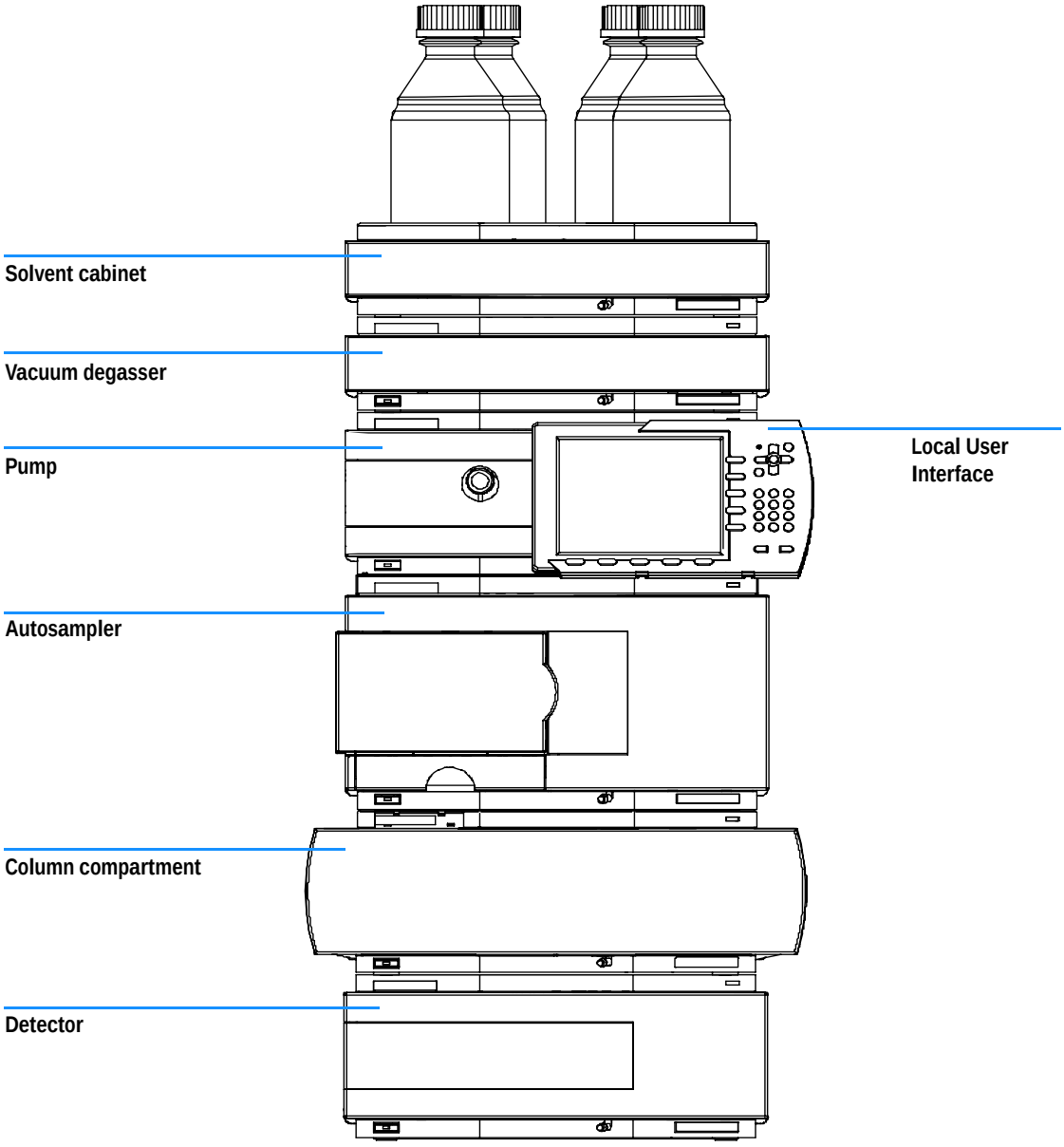


Figure 21 Recommended Stack Configuration (Front View)

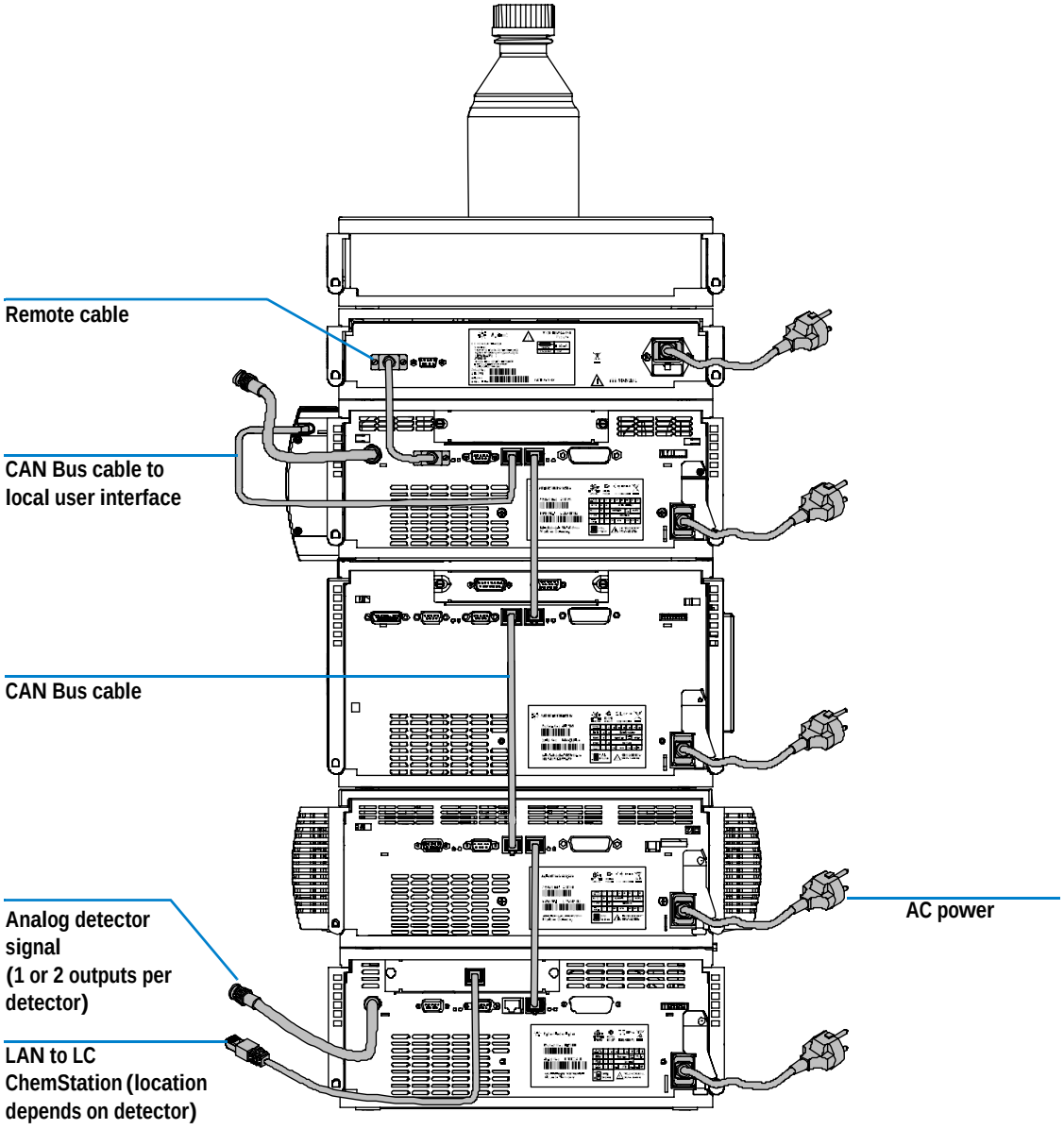

Remote cable
CAN Bus cable to local user interface
CAN Bus cable
Analog detector signal (1 or 2 outputs per detector)
LAN to LC ChemStation (location depends on detector)
AC power

Figure 22 Recommended Stack Configuration (Rear View)

Installing the Detector

Preparations	Locate bench space Provide power connections Unpack the detector
Parts required	Detector Power cord, for other cables see below and "Cable Overview" on page 278, Agilent ChemStation and / or Instant Pilot G4208A or Control Module G1323B.

1 Install the LAN interface board in the detector (if required), see "Replacing the Interface Board" on page 184.

2 Place the detector in the stack or on the bench in a horizontal position.

3 Ensure the line power switch at the front of the detector is OFF.

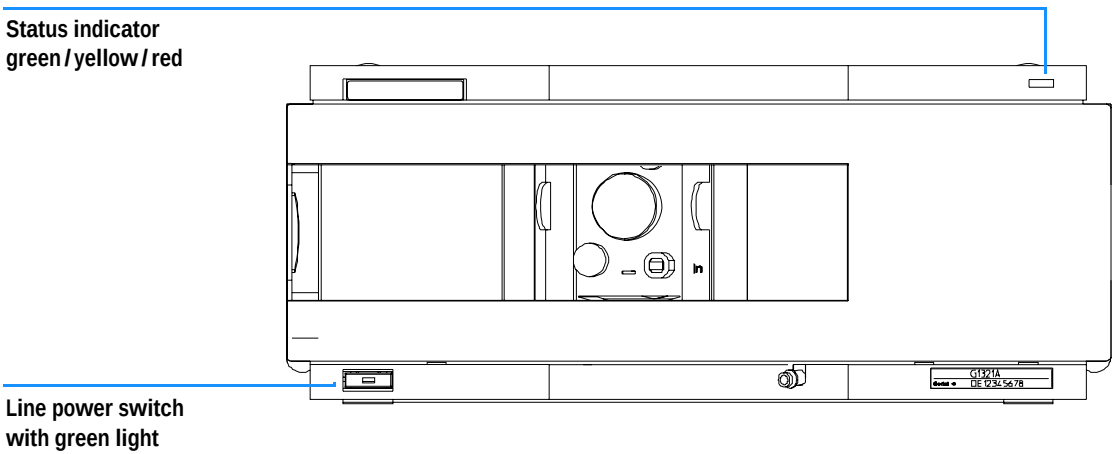


Figure 23 Front View of Detector

4 Connect the power cable to the power connector at the rear of the detector.

5 Connect the CAN cable to other Agilent 1200 Series modules.

6 If an Agilent ChemStation is the controller, connect either
   - the LAN connection to the LAN interface board in the detector.

NOTE If an Agilent 1200 DAD/MWD/FLD is in the system, the LAN should be connected to the DAD/MWD/FLD (due to higher data load).

7 Connect the analog cable(s) (optional).

8 Connect the APG remote cable (optional) for non-Agilent Series instruments.

9 Turn ON power by pushing the button at the lower left hand side of the detector. The status LED should be green.

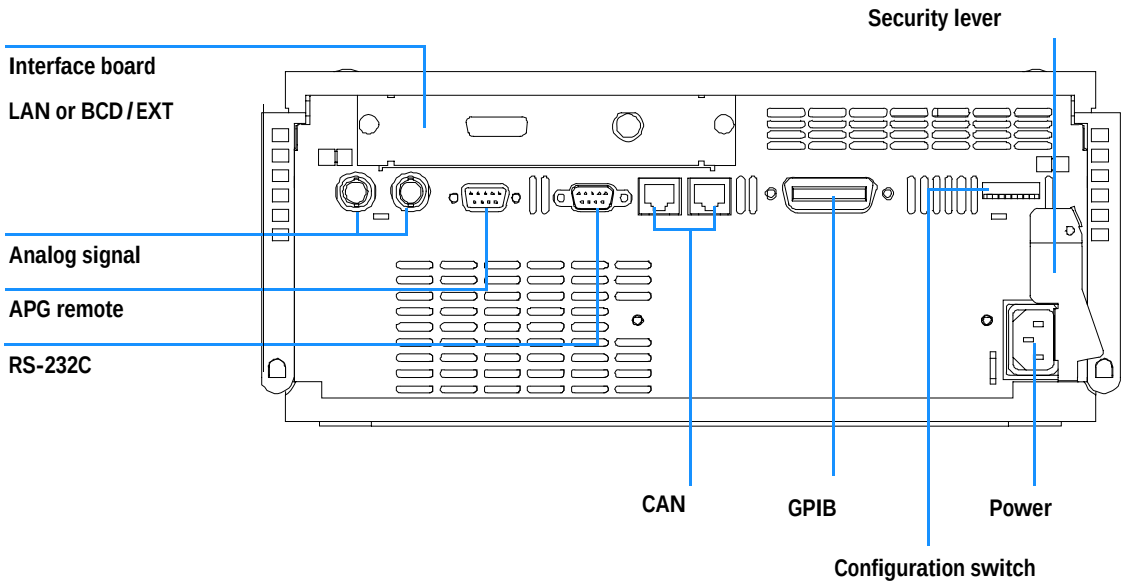


Figure 24 Rear View of Detector

NOTE The detector is turned ON when the line power switch is pressed and the green indicator lamp is illuminated. The detector is turned OFF when the line power switch is protruding and the green light is OFF.

WARNING **To disconnect the detector from line, unplug the power cord. The power supply still uses some power, even if the power switch at the front panel is turned OFF.**

NOTE The detector was shipped with default configuration settings. To change these settings see "Setting the 8-bit Configuration Switch" on page 320.

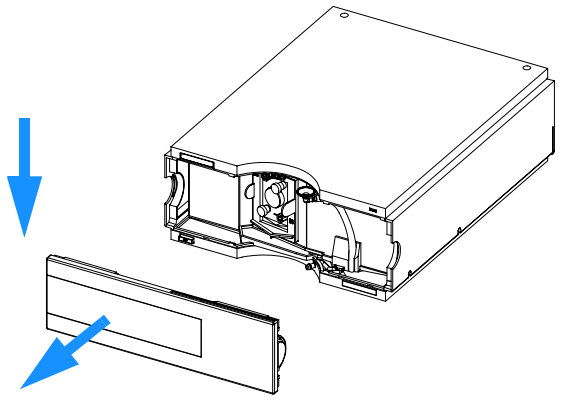
Flow Connections to the Detector

Preparations	Detector is installed in the LC system.
Parts required	Other modules Parts from accessory kit, see"Detector Accessory Kit Contents" on page 49. Two wrenches 1/4 – 5/16 inch for capillary connections

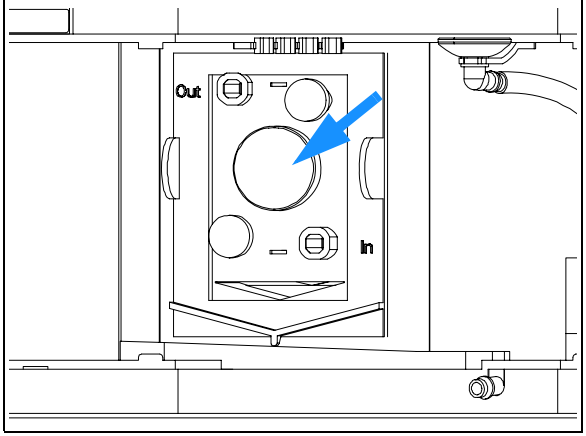
WARNING **When working with solvents please observe appropriate safety procedures (for example, goggles, safety gloves and protective clothing) as described in the material handling and safety data sheet supplied by the solvent vendor, especially when toxic or hazardous solvents are used.**

NOTE The flow cell is shipped with a filling of isopropanol (also recommended when the instrument and/or flow cell is shipped to another location). This is to avoid breakage due to subambient conditions.

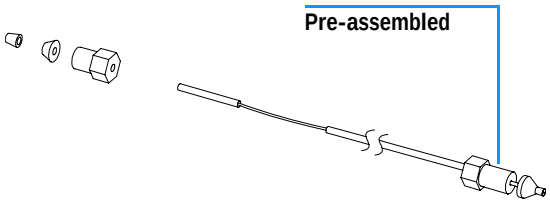
1 Press the release buttons and remove the front cover to gain access to the flow cell area.



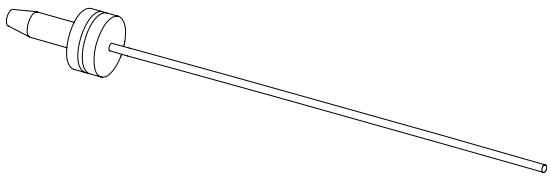
2 Locate the flow cell.



3 Assemble the column detector capillary from the accessory kit. One side is already factory-assembled.



4 Assemble the waste tubing from the accessory kit.



Note:

The fluorescence detector should be the last module in the flow system. An additional detector should be installed before the fluorescence detector to prevent any overpressure to the quartz cell (maximum 20 bar) .

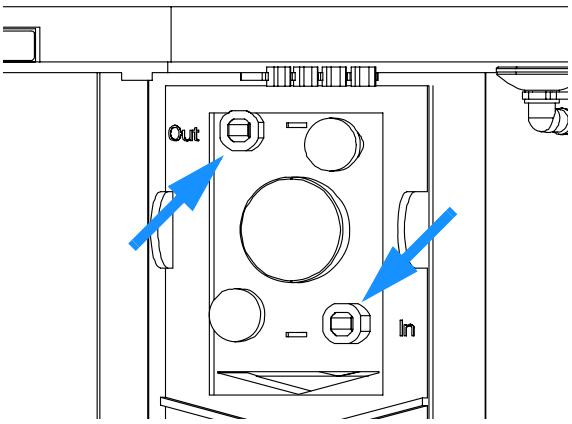
When working with detector behind the FLD (on own risk) determine the backpressure of this detector first by

- removing the column and the last detect and measuring system pressure at the application flow rate.

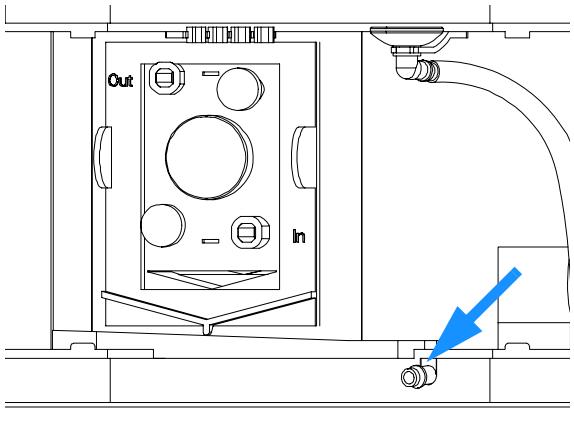
- connecting the last detector (without column and FLD) and measuring the system pressure with flow.

- the difference in measured pressure is due to the back pressure generated by the last detector and is the pressure seen by the FLD.

5 Insert the flow cell and install the capillaries to the flow cell (top is outlet, bottom is inlet).

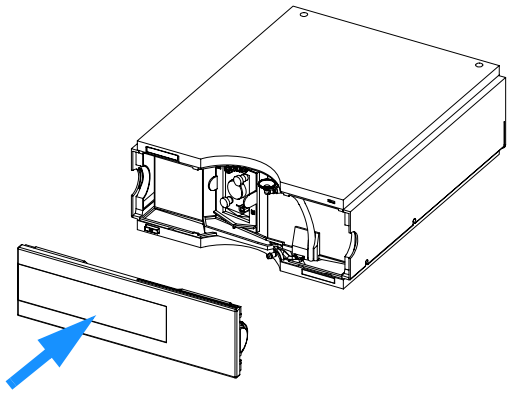


6 Connect the waste tubing to the bottom waste fitting.



7 Establish flow and observe if leaks occur.

8 Replace the front cover.



The installation of the detector is now complete.

NOTE The detector should be operated with the front cover in place to protect the flow cell area against strong drafts from the ouside.

4
First Steps with the Detector

Before You Start 62
Optimization Overview 63
Getting Started and Checkout 65
Method Development 69
Example: Optimization for Multiple Compounds 84

This chapter guides you how to start the work with the detector.

Before You Start

Your normal LC grade solvents usually give good results most of the time. But experience shows that baseline noise can be higher (lower signal-to-noise ratio) when impurities are in the solvents.

Flush your solvent delivery system for at least 15 minutes before checking sensitivity. If your pump has multiple channels, you should also flush the channels not in use.

Optimization Overview

1 Setting the right PMT value

For most applications a setting of 10 is adequate. The G1321A A/D converter exhibits a large linear range making PMT switching unnecessary for most applications. For example, if at high concentrations a peak is cut off; decrease the PMT setting. Remember that low PMT settings decrease the signal to noise ratio.

The built-in PMT gain test uses the parameters in the detector. When using the PMT gain test, the wavelength setting and lamp energy mode (depending on Multiwavelength-Mode and Lamp-Economy) will affect the pmt gain calculation.

NOTE If you have changed one or more parameter(s), you have to press '**OK**' to write down the new settings into the FLD. Then re-enter '**FLD-Signals**' and start the PMT gain test.

2 Using an appropriate response time

For most applications a setting of 4 seconds is adequate. Only for high speed analyses (short columns at high flow rates) a lower setting is recommended. Bear in mind that even if the response time is too high fast peaks will appear a little smaller and broader but retention time and peak areas are still correct and reproducible.

3 Finding the optimum wavelength

Most fluorescent active molecules absorb at 230 nm. Set the excitation wavelength to 230 nm and on-line scan the emission spectra (multi-emission mode). Then set the determined emission wavelength and perform a multi-excitation scan (multi-excitation mode) to find the best excitation wavelength.

4 Evaluating fluorescence spectra

In contrast to diode array based UV detectors where UV spectra are evaluated by taking a spectrum at the peak maximum and selecting a reference spectrum at the baseline, correct fluorescence spectra are obtained by selecting a peak maximum spectrum and a reference around the inflection points. Selecting reference spectra at the baseline is not useful because the spectrum on the baseline is very noisy (no light!).

5 Switching lamp ON only for analysis

Unless maximum sensitivity is needed, the lamp lifetime can significantly be increased by switching it on just for analysis. In contrast to other LC detectors the G1321A fluorescence detector equilibrates within seconds after the lamp is switched ON.

NOTE For highest reproducibility and linearity change the lamp setting to always ON (default is on only during run).
One hour of initial warm-up of the instrument is recommended.

6 Do not overpressurize the detector quartz flow cell

Be aware to not exceed a 20 bar pressure drop after the flow cell when hooking up additional devices like other detectors or a fraction collector. It's better to place a UV detector before the G1321A fluorescence detector.

NOTE When comparing fluorescence excitation spectra directly with DAD spectra or literature based absorbance spectra, you should consider large differences in the used optical bandwidth (FLD=20 nm) which cause a systematic wavelength maximum shift depending on the absorbance spectrum of the compound under evaluation.

Getting Started and Checkout

This chapter describes the check out of the Agilent 1200 Series fluorescence detector using the Agilent isocratic checkout sample.

When required	If you want to checkout the detector
Hardware required	LC system with G1321A FLD
Parts required	Start-up Kit 5063-6528, includes LC cartridge Hypersil ODS, 5um, 125x4mm with CIS cartridge holder Agilent isocratic checkout sample (Part number 01080-68704) Fittings, Qty=2 (Part number 0100-1516) Capillary, 150 mm long, 0.17 mm i.d. (Part number 5021-1817)

Starting Your Detector

1 Turn ON the detector.

2 Turn ON the lamp.

When the lamp is turned on the first time the instrument performs some internal checks and a calibration check which takes about 5 minutes.

3 You are now ready to change the settings of your detector.

Setting the Chromatographic Conditions

1 Set up the system with the following chromatographic conditions and wait until the baseline gets stable.

Table 6 Chromatographic Conditions

Mobile phases	A = water = 35% B = Acetonitrile = 65%
Column	OSD-Hypersil column, 125 mm x 4 mm i.d. with 5 µm particles
Sample	Isocratic standard sample, 1:10 diluted in methanol
Flow rate	1.5 ml/min
Compressibility A (water)	46
Compressibility B (Acetonitrile)	115
Stroke A and B	auto
Stop time	4 minutes
Injection volume	5 µl
Oven temperature (1200)	30°C
FLD Excitations/Emission Wavelength	EX = 246 nm, EM = 317 nm
FLD PMT Gain	PMT = 10
FLD Response time	4 seconds

2 Set the FLD setpoints according to Figure 25 (on the local Control Module G1323B, this information is split across separate screens).

In this example additional excitation wavelengths (B, C, D) are used. This will increase the scan time and may lower the performance.

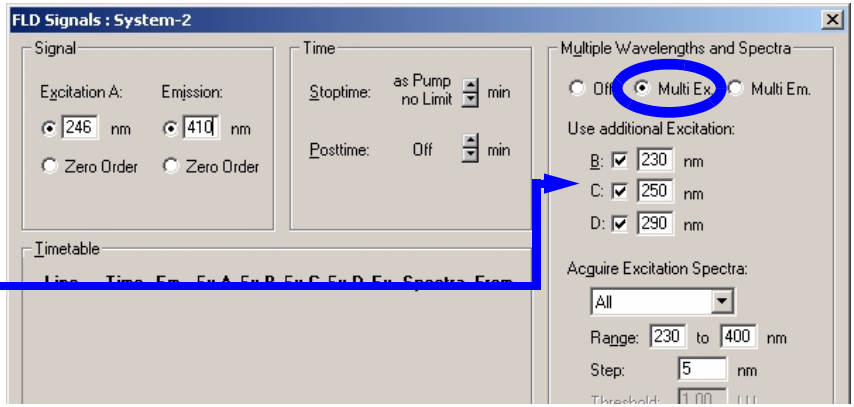


Figure 25 FLD Parameters

3 Start the run.

4 The resulting chromatograms are shown in Figure 26:

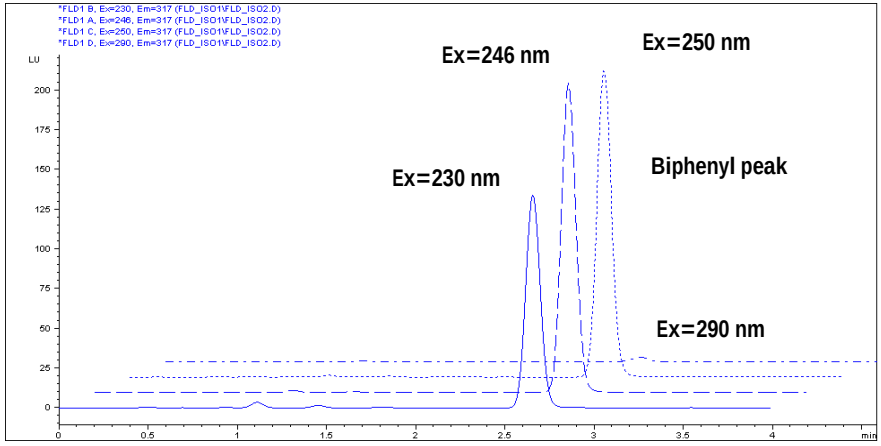


Figure 26 Biphenyl peak with different excitation wavelengths

5 The excitation maxima is around 250 nm.

Observe the maxima via the isoabsorbance plot

1 Load the data file (λ_{EX}=246 nm, λ_{EM}=317 nm) and open the isoabsorbance plot.

2 The maximum λ_{EX} will be found around 250 nm.

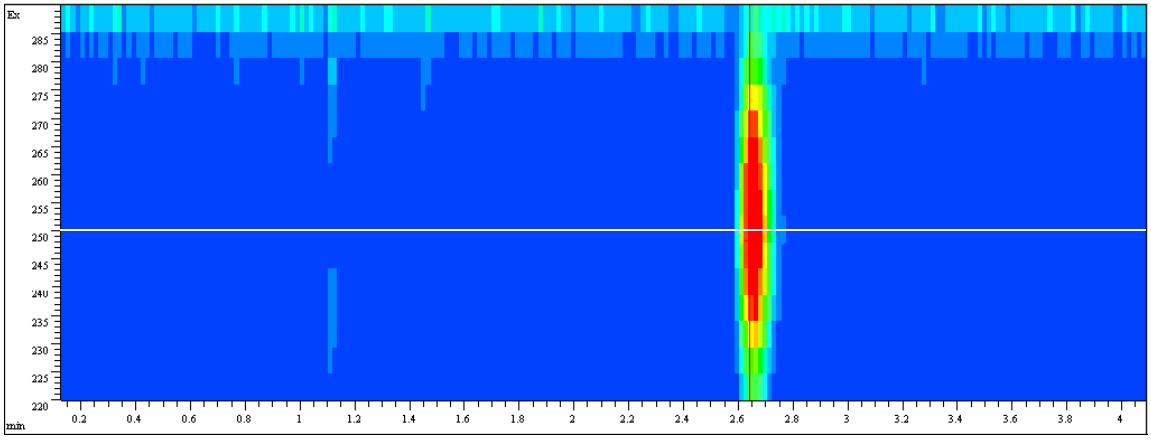


Figure 27 Isoabsorbance Plot

Method Development

Fluorescence detectors are used in liquid chromatography when superior limits of detection and selectivity are required. Thorough method development, including spectra acquisition, is fundamental to achieve good results. This chapter describes three different steps that can be taken with the Agilent 1200 Series fluorescence detector. Table 7 gives an overview of how to benefit from the operation modes during these steps.

Table 7 Steps for thorough method development

	Step 1: Check system	**Step 2: Optimize limits of detection and selectivity**	**Step 3: Set up routine methods**
Fluorescence scan	Find impurities (for example, in solvents and reagents)	Determine simultaneously the excitation and emission spectra of a pure compound	
Signal mode		Perform wavelength switching	Use for lowest limits of detection
Spectral mode / multi-wavelength detection		Determine Ex / Em spectra for all separated compounds in a single run	Collect online spectra, perform library search, determine peak purity
		Activate up to four wavelength simultaneously	Deactivate wavelength switching

Step 1: Check the LC system for impurities

A critical issue in trace level fluorescence detection is to have an LC system free of fluorescent contamination. Most contaminants derive from impure solvents. Taking a fluorescence scan is a convenient way to check the quality of the solvent in a few minutes. This can be done, for example, by filling the FLD cuvette directly with the solvent for an offline measurement even before the start of a chromatographic run. The result can be displayed as an isofluorescence plot or a three-dimensional plot. Different colors reflect different intensities.

Figure 28 shows a sample of slightly impure water which was planned for use as mobile phase. The area where fluorescence of the contaminated water sample can be seen is between the stray light areas: the first- and second-order Raleigh stray light and Raman stray light.

A pure water sample was put into the flow cell. Spectra were recorded at 5 nm step sizes.

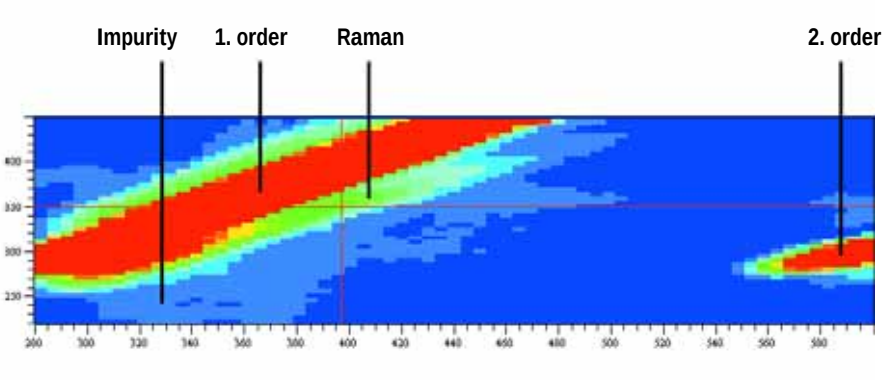


Figure 28 Isofluorescence plot of a mobile phase

Since "excitation" and "emission" wavelength are the same for Raleigh stray light, the area of first-order Raleigh stray light is visible in the left upper area of the diagram. The Raman bands of water are seen below the first-order Raleigh stray light. Since the cut-off filter cuts off light below 280 nm, the second-order Raleigh stray light starts above 560 nm.

Stray light acts in the same way as impurities in that it simulates background noise. In both cases, a higher noise level and therefore a higher limit of detection are obtained. This indicates that high sensitivity measurements should be done away from wavelength settings that have a high stray light background.

Step 2: Optimize limits of detection and selectivity

To achieve optimum limits of detection and selectivity, analysts must find out about the fluorescent properties of the compounds of interest. Excitation and emission wavelengths can be selected for optimum limits of detection and best selectivity. In general, fluorescence spectra obtained with different instruments may show significant differences depending on the hardware and software used.

The traditional approach is to extract an appropriate excitation wavelength from the UV spectrum that is similar to the fluorescence excitation spectrum (see Figure 29) and to record the emission spectrum. Then with an optimum emission wavelength determined, the excitation spectrum is acquired.

Excitation spectrum with emission at 440 nm, emission spectrum with excitation at 250 nm of 1 ug / ml quinidine.

Detector settings: step size 5 nm, PMT 12, Response time 4 s.

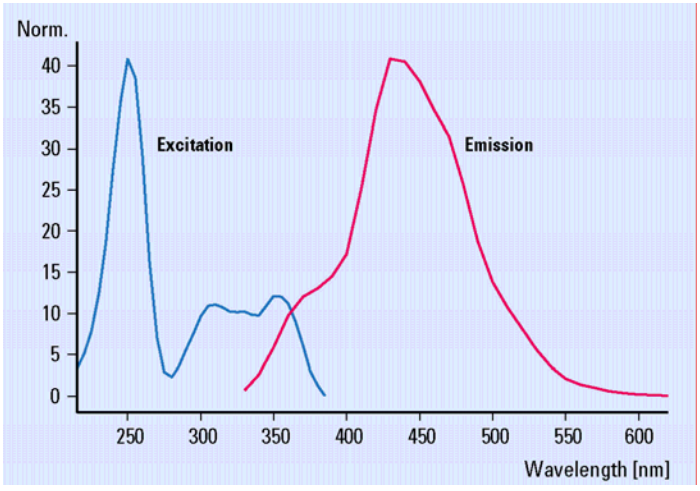


Figure 29 Excitation and emission spectra of quinidine

These tasks have to be repeated for each compound using either a fluorescence spectrophotometer or stop-flow conditions in LC. Usually each compound requires a separate run. As a result, a set of excitation and emission spectrum is obtained (Figure 28 on page 70) for each compound. Since this is a tedious procedure, it is applicable only when there is a limited number of compounds of interest.

The Agilent 1200 Series LC offers three different ways to obtain complete information on a compound's fluorescence:

Procedure I - Take a fluorescence scan offline for a single compound as described above for the mobile phase. This is done preferably with a manual FLD cuvette when pure compounds are available.

Procedure II - Use two LC runs with the Agilent 1200 Series FLD to separate the compound mix under known conditions and acquire emission and excitation spectra separately.

Procedure III - Use an Agilent 1200 Series FLD/DAD combination and acquire UV/Visible spectra (equivalent to excitation spectra) with the DAD and emission spectra with the FLD-both in a single run.

Procedure I - Take a fluorescence scan

Because fluorescence spectra traditionally have not been easily available with previous LC fluorescence detectors, standard fluorescence spectrophotometers have been used in the past to acquire spectral information for unknown compounds. Unfortunately this approach limits optimization, as there are differences expected in optical design between an LC detector and a dedicated fluorescence spectrophotometer, or even between detectors. These differences can lead to variations for the optimum excitation and emission wavelengths.

The Agilent 1200 Series fluorescence detector offers a fluorescence scan that delivers all spectral information previously obtained with a standard fluorescence spectrophotometer, independent of the LC fluorescence detector. Figure 30 on page 74 shows the complete information for quinidine as obtained with the Agilent 1200 Series FLD and a manual cuvette in a single offline measurement. The optima for excitation and emission wavelengths can be extracted as coordinates of the maxima in the three dimensional plot. One of the three maxima in the center of the plot can be chosen to define the excitation wavelength. The selection depends on the additional compounds

that are going to be analyzed in the chromatographic run and the background noise that may be different upon excitation at 250 nm, 315 nm or 350 nm. The maximum of emission is observed at 440 nm.

Details for Figure 30 on page 74:

All excitation and emission spectra of Quinidine (1 µg/ml) are shown in graphic. Fluorescence intensity is plotted vs excitation and emission wavelengths.

Detector settings: step size 5 nm, PMT 12, Response time 4 s

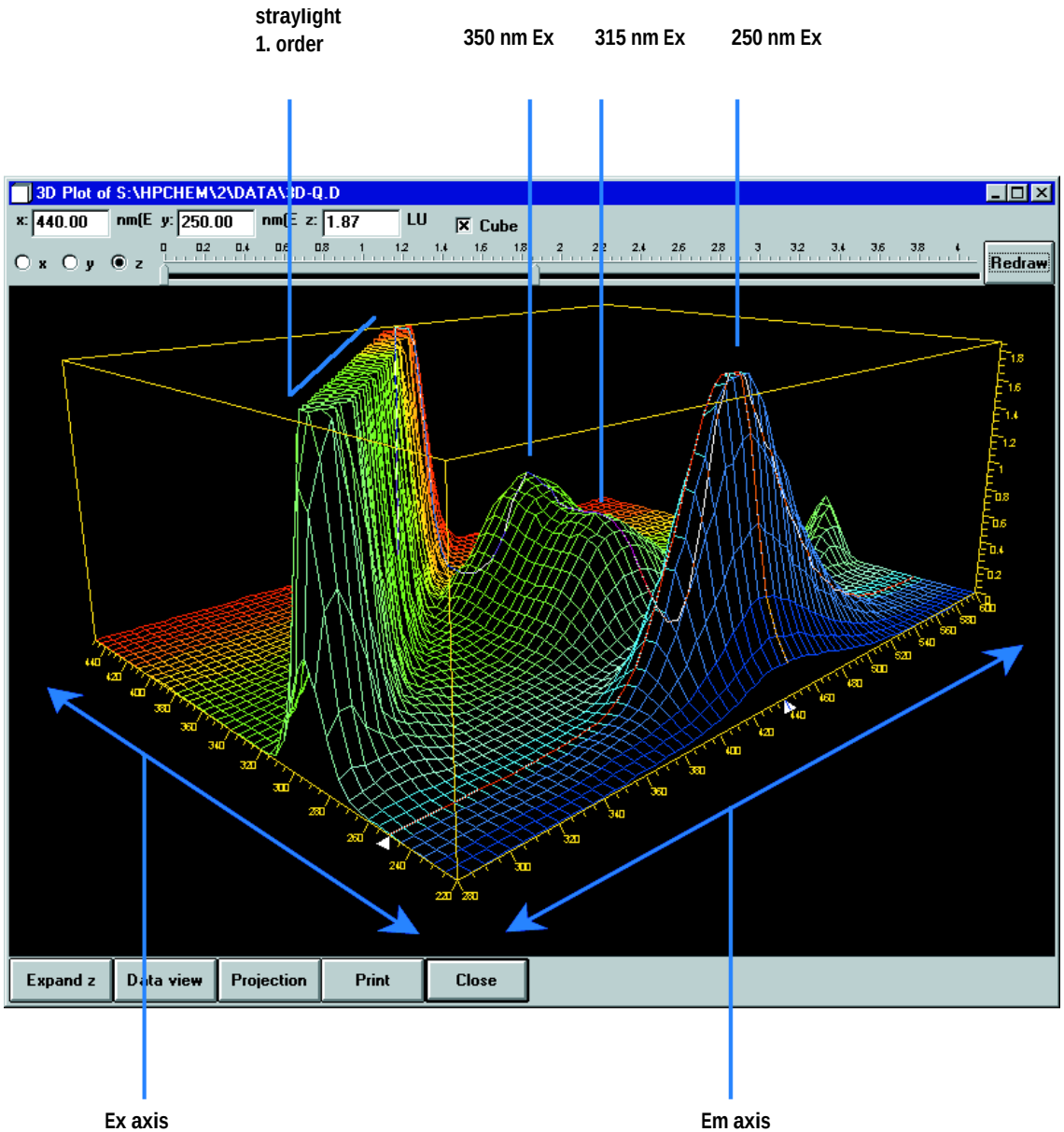


Figure 30 Characterization of a pure compound from a fluorescence scan

Procedure II - Take two LC runs with the FLD

The conditions for the separation of organic compounds such as polyaromatic nuclear hydrocarbons (PNAs) are well described in various standard methods, including commonly used EPA and DIN methods. Achieving the best detection levels requires checking for the optimum excitation and emission wavelengths for all compounds. Yet taking fluorescence scans individually makes this a tedious process. A better approach is to acquire spectra online for all compounds during a run. This speeds up method development tremendously. Two runs are sufficient for optimization.

During the *first run*, one wavelength is chosen in the low UV range for the excitation wavelength and one emission wavelength in the spectral range for the emission wavelength. Most fluorophores show strong absorption at these wavelengths and the quantum yield is high. Excitation is sufficient for collecting emission spectra.

Figure 31 on page 76 contains all emission spectra obtained in a single run from a mix of 15 PNAs. This set of spectra is used to set up a timetable for optimum emission wavelengths for all compounds.

The individual compound spectra in the isofluorescence plot show that at least three emission wavelengths are needed to detect all 15 PNAs properly:

Table 8 Timetable for PNA analysis

0 min:	350 nm	for naphthalene to phenanthrene
8.2 min:	420 nm	for anthracene to benzo(g,h,I)perylene
19.0 min:	500 nm	for indeno(1,2,3-cd)pyrene

In the second run, three setpoints for emission wavelengths are entered into the time-program and excitation spectra are recorded, as shown in figure 8. The area of high intensity (red) is caused by stray light when emission spectra overlap with the excitation wavelength. This can be avoided by fitting the spectral range automatically. Excitation at 260 nm is most appropriate for all PNAs.

Table 9 Conditions for Figure 31 and Figure 32 on page 77

Column	Vydac, 2.1 x 200 mm, PNA, 5 µm
Mobile phase	A = water; B = acetonitrile (50:50)
Gradient	3 minutes, 60% 14 minutes, 90% 22 minutes, 100%
Flow rate	0.4 ml/min
Column temperature	18 ° C
Injection volume	5 µl
FLD settings	PMT 12, response time 4 s, step size 5 nm

This shows the isofluorescence plot of emission spectra for 15 PNAs (5 µg/ml) with a fixed excitation wavelengths (260 nm).

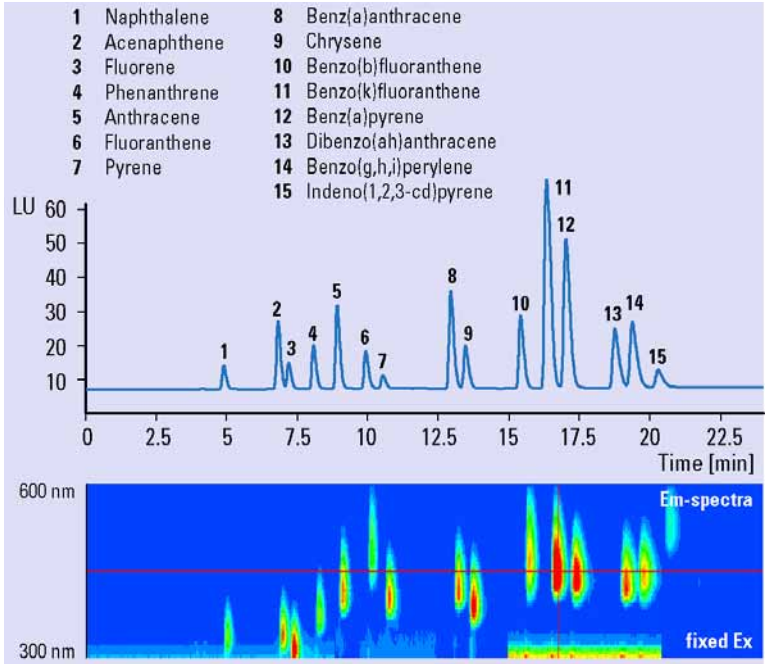


Figure 31 Optimization of the time-program for the emission wavelength

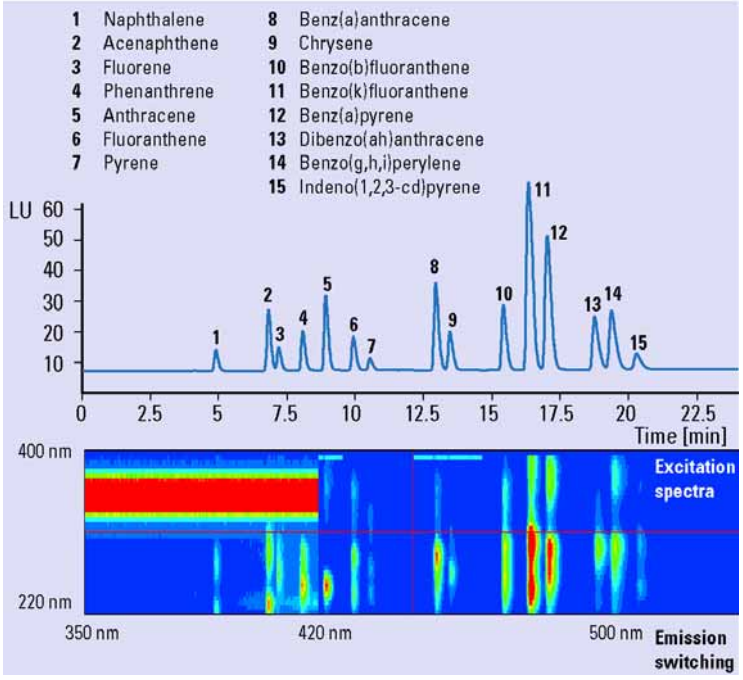


Figure 32 Optimization of the time-program for the excitation wavelength

The obtained data are combined to setup the time-table for the excitation wavelength for best limit of detection and selectivity. The optimized switching events for this example are summarized in Table 10.

Table 10 Timetable for the analysis of 15 polynuclear aromatic hydrocarbons

Time [min]	Exitation Wavelength [nm]	Emission Wavelength [nm]
0	260	350
8.2	260	420
19.0	260	500

This timetable gives the conditions for optimum detection based on the results of two chromatographic runs.

Procedure III - Make a single run with the Agilent 1200 Series DAD / FLD combination

For most organic compounds, UV-spectra from diode array detectors are nearly identical to fluorescence excitation spectra. Spectral differences are caused by specific detector characteristics such as spectral resolution or light sources.

In practice, combining a diode array detector with a fluorescence detector in series gives the full data set needed to achieve the optimum fluorescence excitation and emission wavelengths for a series of compounds in a single run. With the UV/Visible/excitation spectra available from the diode array detector, the fluorescence detector is set to acquire emission spectra with a fixed excitation wavelength in the low UV range.

The example is taken from the quality control of carbamates. Samples are analyzed for the impurities 2,3-diamino-phenazine (DAP) and 2-amino-3-hydroxyphenazine (AHP). Reference samples of DAP and AHP were analyzed with diode array and fluorescence detection. Figure 9 shows the spectra obtained from both detectors for DAP. The excitation spectrum of DAP is very similar to the UV absorption spectrum from the diode array detector. Figure 34 on page 79 shows the successful application of the method to a carbamate sample and a pure mixture of DAP and AHP for reference. The column was overloaded with the non-fluorescent carbamate (2-benzimidazole carbamic acid methylester/MBC) to see the known impurities, AHP and DAP.

This is an impurity of carbamates. The excitation spectrum in a second run shows the equivalence of UV-spectra and fluorescence excitation spectra. An excitation wavelength at 265 nm was used for taking the emission spectrum and an emission wavelength at 540 nm was used for taking the excitation spectrum.

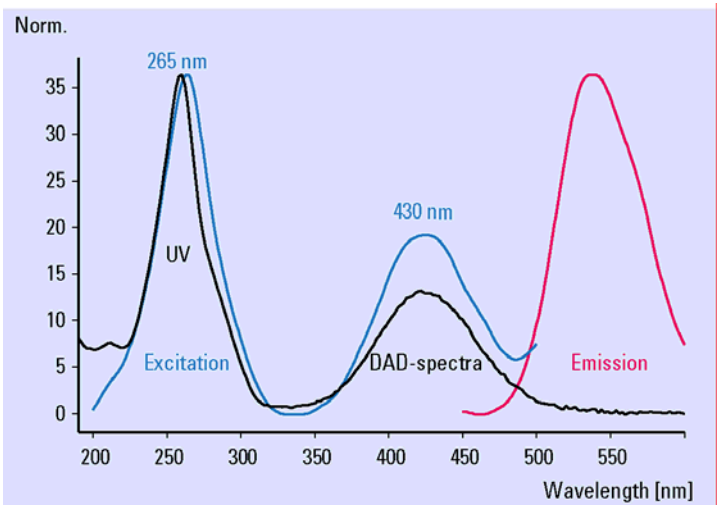


Figure 33 UV-spectrum and fluorescence spectra for 2,3-diaminophenazine (DAP)

The two upper traces are obtained using two different excitation wavelengths. The lower trace is a pure standard of the known impurities.

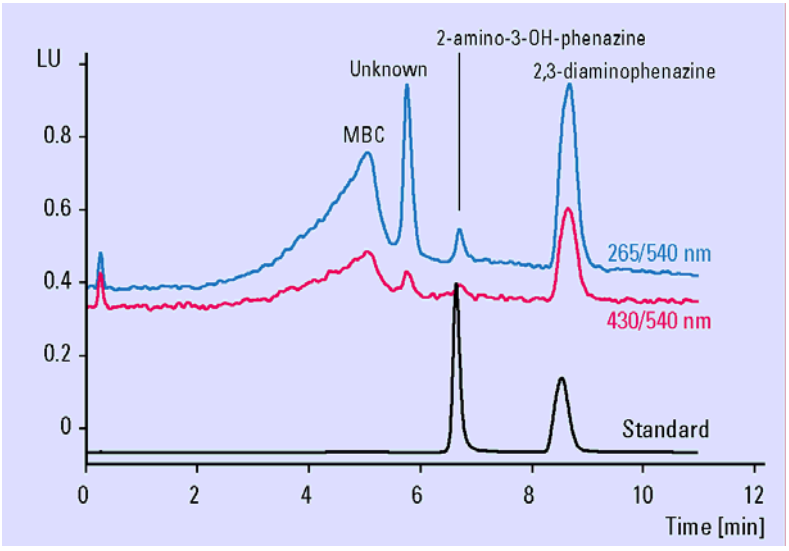


Figure 34 Qualitive analysis of MBC (2-benzimidazole carbamic acid methylester) and impurities

Table 11 Conditions for Figure 33 and Figure 34 on page 79

Column	Zorbax SB, 2 x 50 mm, PNA, 5 µm
Mobile phase	A = water; B = acetonitrile
Gradient	0 minutes, 5% 10 minutes, 15%
Flow rate	0.4 ml/min
Column temperature	35 ° C
Injection volume	5 µl
FLD settings	PMT 12, response time 4 s, step size 5 nm Ex 265 nm and 430 nm Em 540 nm

Step 3: Set up routine methods

In routine analysis, sample matrices can have a significant influence on retention times. For reliable results, sample preparation must be thorough to avoid interferences or LC methods must be rugged enough. With difficult matrices, simultaneous multi-wavelength detection offers more reliability than timetable-controlled wavelength switching. The Agilent 1200 Series FLD can, in addition, acquire fluorescence spectra while it records the detector signals for quantitative analysis. Therefore qualitative data are available for peak confirmation and purity checks in routine analysis.

Multi wavelength detection

Time-programmed wavelength switching traditionally is used to achieve low limits of detection and high selectivity in routine quantitative analysis. Such switching is difficult

if compounds elute closely and require a change in excitation or emission wavelength. Peaks can be distorted and quantitation made impossible if wavelength switching occurs during the elution of a compound. Very often this happens with complex matrices, influencing the retention of compounds.

In spectral mode, the Agilent 1200 Series FLD can acquire up to four different signals simultaneously. All of them can be used for quantitative analysis. Apart from complex matrices, this is advantageous when watching for impurities at additional wavelengths. It is also advantageous for reaching low limits of detection or increasing selectivity through optimum wavelength settings at any time. The number of data points acquired per signal is reduced and thus limits of detection may be higher, depending on the detector settings compared to the signal mode.

PNA analysis, for example, can be performed with simultaneous multi wavelength detection instead of wavelength-switching. With four different wavelengths for emission, all 15 PNAs can be monitored (Figure 35 on page 82).

Table 12 Conditions for Figure 35 on page 82

Column	Vydac, 2.1 x 250 mm, PNA, 5 µm
Mobile phase	A = water; B = acetonitrile (50:50)
Gradient	3 minutes, 60% 14.5 minutes, 90% 22.5 minutes, 95%
Flow rate	0.4 ml/min
Column temperature	22 ° C
Injection volume	2 µl
FLD settings	PMT 12, response time 4 s,

The upper trace was received with traditional wavelength switching.

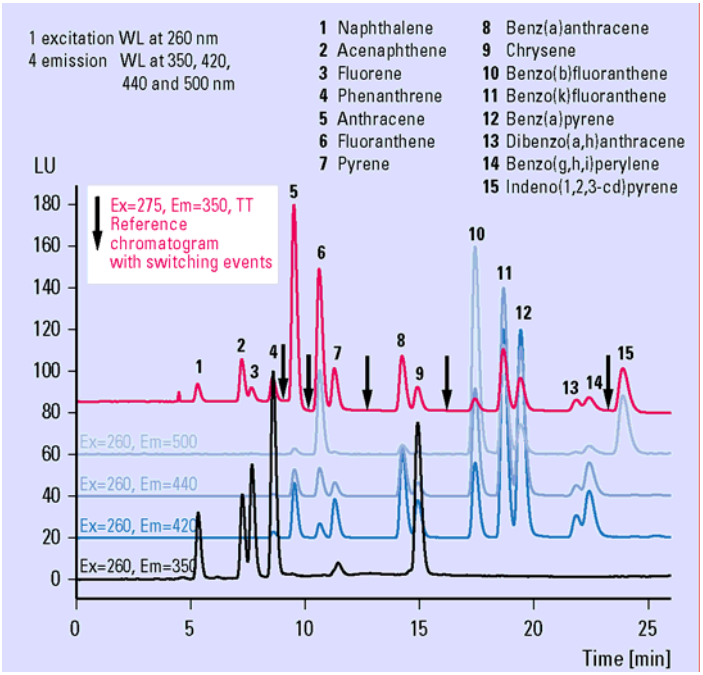


Figure 35 Simultaneous multi wavelength detection for PNA-analysis

Previously, only diode array detectors and mass spectrometric detectors could deliver spectral information on-line to confirm peak identity as assigned by retention time.

Now, fluorescence detectors provide an additional tool for automated peak confirmation and purity control. No additional run is necessary after the quantitative analysis.

During method development, fluorescence excitation and emission spectra are collected from reference standards and entered into a library-at the choice of the method developer. All spectral data from unknown samples can then be compared automatically with library data. Table 3 illustrates this principle using a PNA analysis. The match factor given in the report for each peak indicates the degree of similarity between the reference spectrum and the spectra from a peak. A match factor of 1,000 means identical spectra.

In addition, the purity of a peak can be investigated by comparing spectra obtained within a single peak. When a peak is calculated to be within the user-defined purity limits, the purity factor is the mean purity value of all spectra that are within the purity limits.

The reliability of the purity and the match factor depends on the quality of spectra recorded. Because of the lower number of data points available with the fluorescence detector in general, the match factors and purity data obtained show stronger deviations compared to data from the diode array detector, even if the compounds are identical.

Table 13 shows an automated library search based on the emission spectra from a PNA reference sample.

Table 13 Peak confirmation using a fluorescence spectral library

Meas. RetTime [min]	Library RetTime [min]	CalTbl [min]	Signal	Amount [ng]	Purity Factor	#	Match	Libary Name
4.859	4.800	5.178	1	1.47986e-1	-	1	993	Naphthalene@em
6.764	7.000	7.162	1	2.16156e-1	-	1	998	Acenaphthene@em
7.137	7.100	7.544	1	1.14864e-1	-	1	995	Fluorene@em
8.005	8.000	8.453	1	2.56635e-1	-	1	969	Phenanthrene@em
8.841	8.800	9.328	1	1.76064e-1	-	1	993	Anthracene@em
9.838	10.000	10.353	1	2.15360e-1	-	1	997	Fluoranthene@em
10.439	10.400	10.988	1	8.00754e-2	-	1	1000	Pyrene@em
12.826	12.800	13.469	1	1.40764e-1	-	1	998	Benz(a)anthracene@em
13.340	13.300	14.022	1	1.14082e-1	-	1	999	Chrysene@em
15.274	15.200	16.052	1	6.90434e-1	-	1	999	Benzo(b)fluoranthene@em
16.187	16.200	17.052	1	5.61791e-1	-	1	998	Benzo(k)fluoranthene@em
16.865	16.900	17.804	1	5.58070e-1	-	1	999	Benz(a)pyrene@em
18.586	18.600	19.645	1	5.17430e-1	-	1	999	Dibenz(a,h)anthracene@em
19.200	19.100	20.329	1	6.03334e-1	-	1	995	Benzo(g,h,i)perylene@em
20.106	20.000	21.291	1	9.13648e-2	-	1	991	Indeno(1,2,3-cd)pyrene@em

Example: Optimization for Multiple Compounds

Using PNAs as a sample, this example uses the described scanning functions.

Setting the Chromatographic Conditions

1 This example uses the following chromatographic conditions (the detector settings are shown in Figure 36 on page 85).

Table 14 Chromatographic Conditions

Mobile phases	A = water = 50% B = Acetonitrile = 50%
Column	Vydac-C18-PNA, 250 mm x 2.1 mm i.d. with 5 µm particles
Sample	PAH 0.5 ng
Flow rate	0.4 ml/min
Compressibility A (water)	46
Compressibility B (Acetonitrile)	115
Stroke A and B	auto
Time Table	at 0 minutes %B=50
	at 3 minutes %B=60
	at 14.5 minutes %B=90
	at 22.5 minutes %B=95
Stop time	26 minutes
Post time	8 minutes
Injection volume	1 µl
Oven temperature (1200)	30°C
FLD PMT Gain	PMT = 15
FLD Response time	4 seconds

Select a Excitation wavelength in the low UV (230...260 nm). This will cover nearly all fluorescence in your sample.

DO NOT select additional emission wavelengths (B, C, D). Doing so will increase the scan time and will lower the performance.

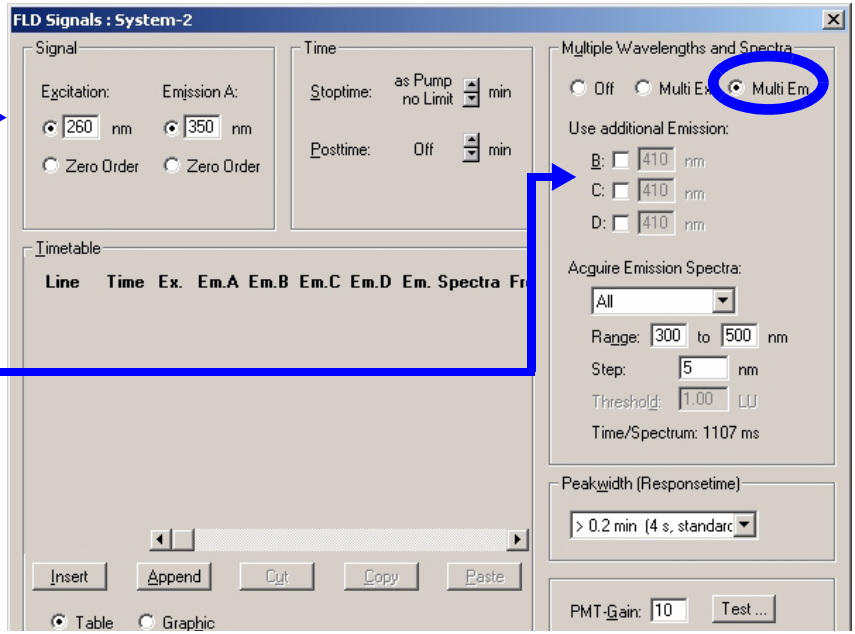


Figure 36 Detector Settings for Emission Scan

2 Wait until the baseline stabilizes. Complete the run.

3 Load the signal. (In this example just the time range of 13 minutes is displayed).

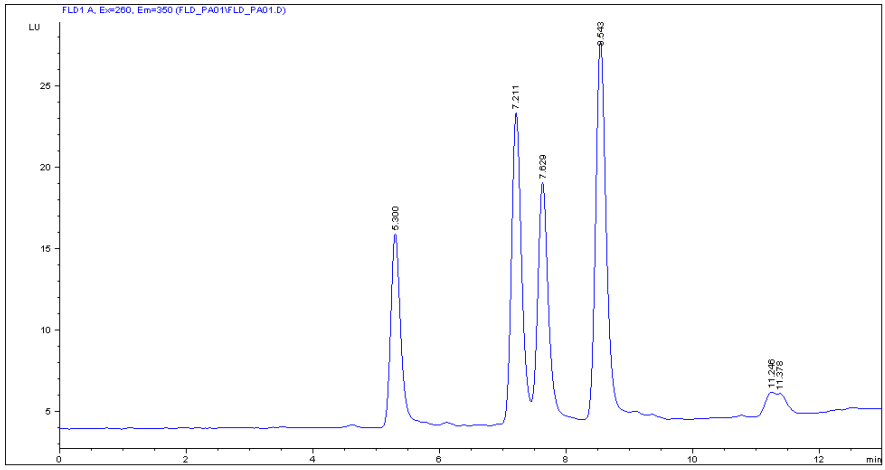


Figure 37 Chromatogram from Emissions Scan

4 Use the isoabsorbance plot and evaluate the optimal emission wavelengths, shown in the table below.

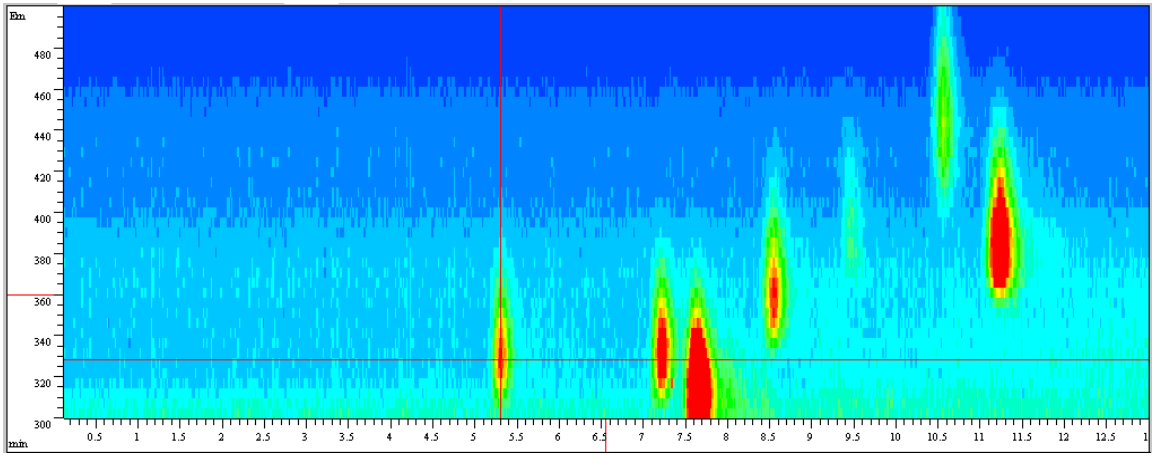


Figure 38 Isoabsorbance Plot from Emission Scan

Table 15

Peak #	Time	Emission Wavelength
1	5.3 min	330 nm
2	7.2 min	330 nm
3	7.6 min	310 nm
4	8.6 min	360 nm
5	10.6 min	445 nm
6	**11.23 min**	385 nm

5 Using the settings and the timetable (from previous page), do a second run for the evaluation of the optimal excitation wavelength. See Figure 39.

DO NOT select additional excitation wavelengths (B, C, D). Doing so will increase the scan time and will lower the performance

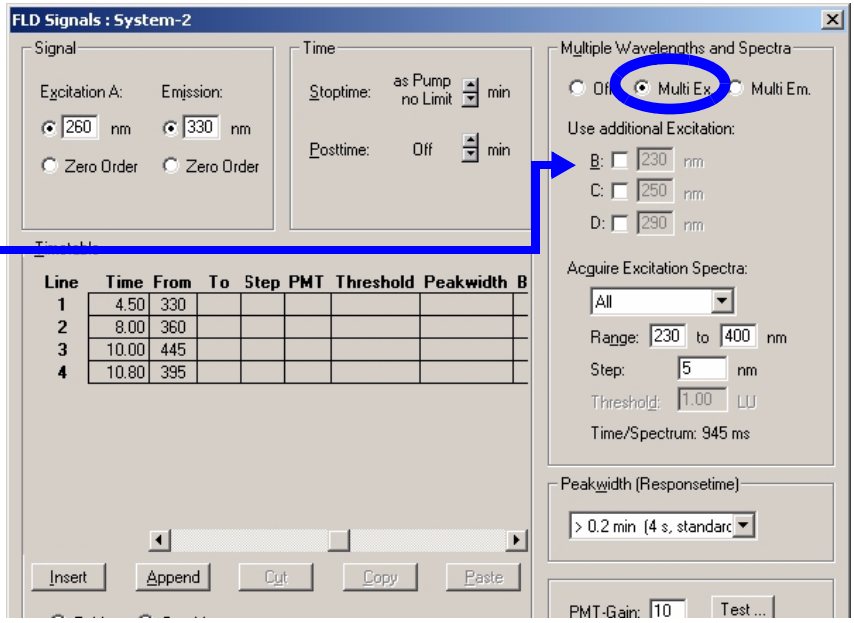


Figure 39 Detector Settings for Excitation Scan

6 Wait until the baseline stabilizes. Start the run.

7 Load the signal.

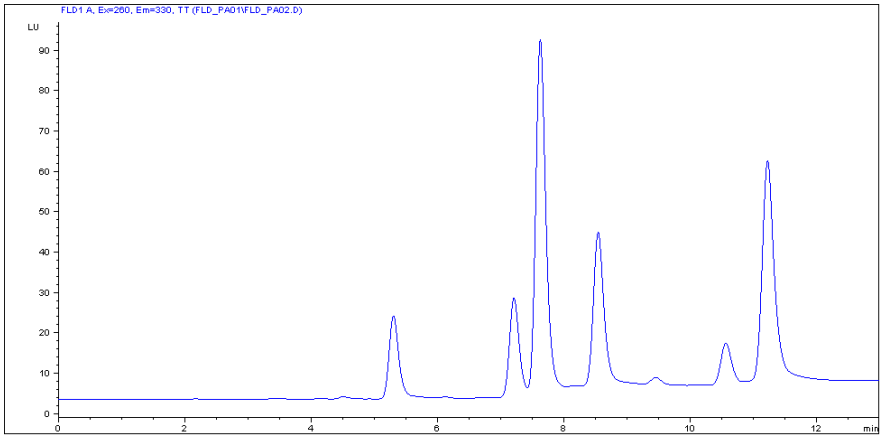


Figure 40 Chromatogram - excitation scan at reference wavelength 260/330 nm

8 Use the isoabsorbance plot and evaluate the optimal excitation wavelengths (in this example just in the time range of 13 minutes).

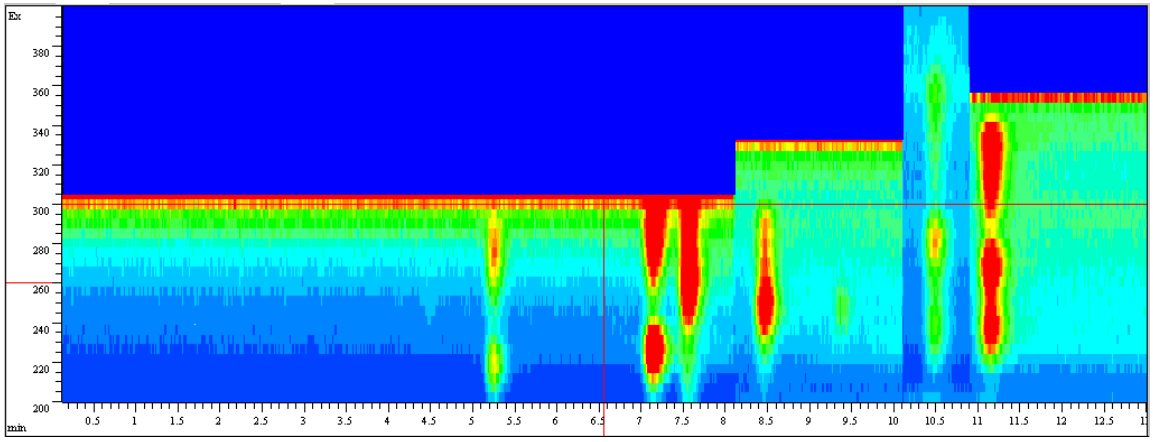


Figure 41 Isoabsorbance Plot - Excitation

The table below shows the complete information about emission (from Figure 38 on page 86) and excitation maxima.

Table 16

Peak #	Time	Emission Wavelength	Excitation Wavelength
1	5.3 min	330 nm	220 / 280 nm
2	7.3 min	330 nm	225 / 285 nm
3	7.7 min	310 nm	265 nm
4	8.5 min	360 nm	245 nm
5	10.7 min	445 nm	280 nm
6	**11.3 min**	385 nm	270 / 330 nm

Evaluating The System Background

The example below uses water.

1 Pump solvent through your system.

2 Set the fluorescence scan range under FLD special setpoints according to your needs.

NOTE The scan time will increase when the range is enlarged. With the default values, the scan takes about 2 minutes.

3 Set PMT gain to 16.

The wavelength range and step number defines the duration. Using the maximum range, the scan would take approximately 10 minutes

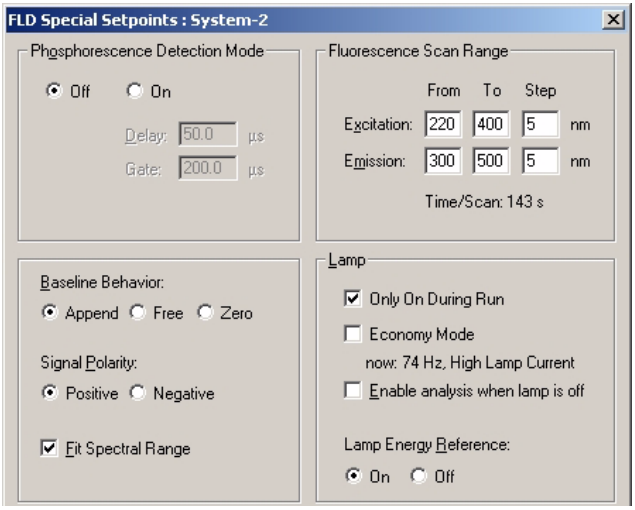


Figure 42 FLD special settings

4 Define a data file name and take a fluorescence scan. After the scan is completed, the isoabsorbance scan results appear, see Figure 43 on page 90.

NOTE A low background will improve the signal-to-noise, see also "Reducing Stray Light" on page 102.

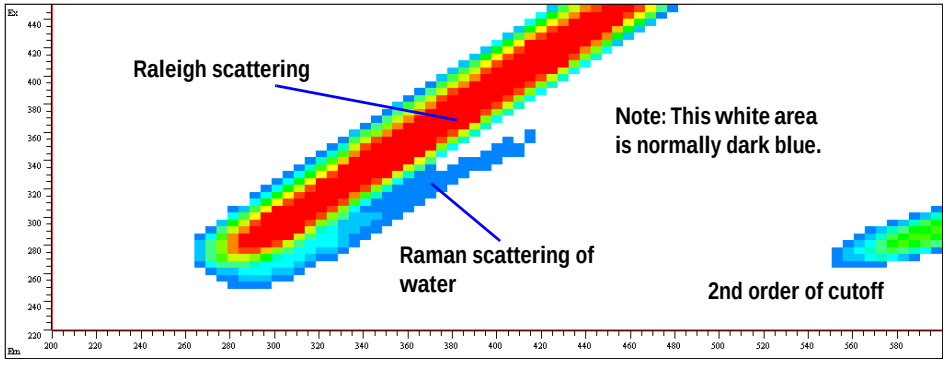


Figure 43 Fluorescence Scan of Water

5
How to optimize the Detector

Optimization Overview 92
Design Features Help Optimization 93
Finding the Best Wavelengths 94
Finding the Best Signal Amplification 96
Changing the Xenon Flash Lamp Frequency 98
Selecting the Best Response Time 100
Reducing Stray Light 102

This chapter provides information on how to optimize the detector.

Optimization Overview

Refer to “Optimization Overview” on page 63 for details.

Design Features Help Optimization

The Agilent 1200 Series fluorescence detector has several features you can use to optimize detection:

Table 17

PMTGAIN	Amplification factor
LAMP	Flash frequency
RESPONSETIME	Data reduction interval

Check Performance Before You Start

Before you start you should check that your detector is performing according to the specifications published by Agilent Technologies.

Your normal LC grade solvents may give good results most of the time but our experience shows that baseline noise can be higher with LC grade solvents than with fluorescence grade solvents.

Flush your solvent delivery system for at least 15 minutes before checking sensitivity. If your pump has multiple channels, you should also flush the channels not in use.

Finding the Best Wavelengths

The most important parameters to be optimized in fluorescence detection are the excitation and emission wavelengths. Generally, it is assumed that the best excitation wavelength can be taken from the excitation spectrum acquired on a spectrofluorimeter. It is also assumed that once the optimal excitation wavelength has been found for one particular instrument type this wavelength can also be applied to other instruments.

Both assumptions are wrong.

The optimum wavelength for the excitation depends on the absorption of the compounds but also on the instrument characteristics, for example the lamp type and the gratings. As most organic molecules absorb best in the ultra-violet range the Agilent 1200 Series fluorescence detector was designed to give an optimum signal-to-noise ratio in the 210 nm to 360 nm range of the spectrum. To achieve greatest sensitivity, the absorbance wavelength of your sample molecule should match the wavelength range for your instrument. In other words, an excitation wavelength in the ultra-violet range. Your Agilent 1200 Series fluorescence detector has a broad excitation wavelength range, but for higher sensitivity you should choose a wavelength in the ultra-violet range (near 250 nm).

The design elements that contribute to lower efficiency in the lower ultra-violet range are the xenon flash lamp and the gratings. Flash-type lamps shift the optimum wavelength to lower wavelength ranges with the Agilent 1200 Series fluorescence detector to a maximum of 250 nm. The excitation grating is blazed for highest efficiency at 300 nm.

A Real Example

Although an excitation wavelength of 340 nm is quoted in the literature the Agilent 1200 Series fluorescence detector scan of orthophthalaldehyde, a derivative of the amino acid alanine, (Figure 44 on page 95) shows a maximum between 220 nm and 240 nm.

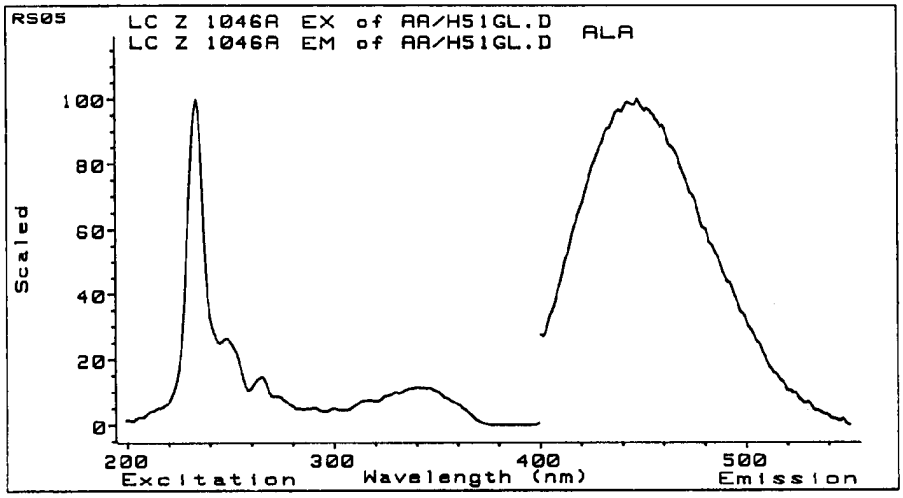


Figure 44 Scan Orthophthalaldehyde Derivative of Alanine

When you are looking for the wavelength by scanning, scan over the whole range. As this example shows a maximum may be found in a completely different wavelength range.

NOTE

When comparing fluorescence excitation spectra directly with DAD spectra or literature based absorbance spectra, you should consider large differences in the used optical bandwidth (FLD=20 nm) which cause a systematic wavelength maximum shift depending on the absorbance spectrum of the compound under evaluation.

Finding the Best Signal Amplification

Increasing the PMTGAIN increases the signal and the noise. Up to a certain factor the increase in signal is higher than the increase in noise.

The step from gain to gain is equal to a factor of 2 (which is the same as on the HP 1046A FLD).

In Figure 45 the PMTGAIN was gradually raised from 4 up to 11 (the peak is from the Agilent Technologies isocratic sample which was diluted 1000 times). With increasing PMTGAIN there was an improvement in signal-to-noise up to 10. Above 10 the noise increased proportionately to the signal with no improvement in signal-to-noise.

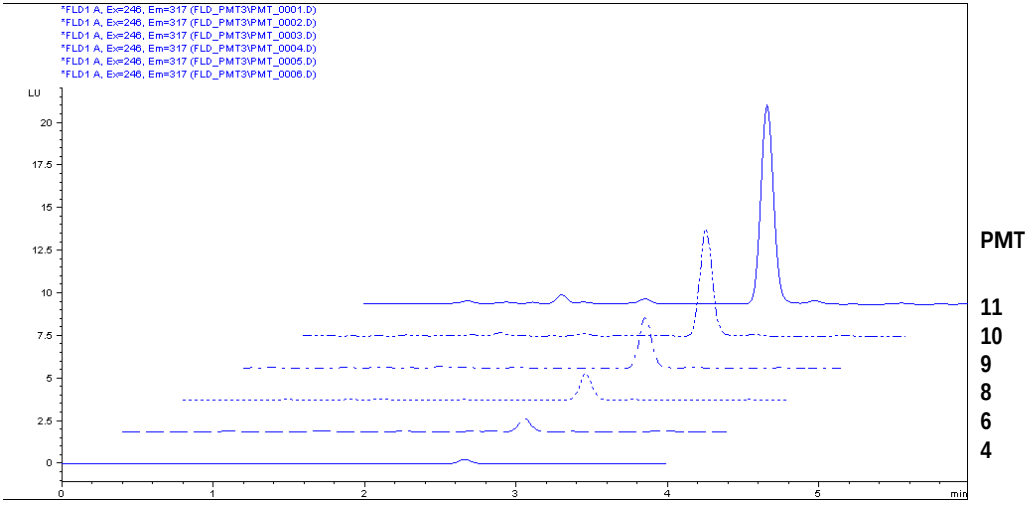


Figure 45 Finding Best PMTGAIN for Biphenyl

The reason for this is the fact, that quantification of baselines (especially at low background levels) is not sufficient for statistically working filter methods. For the best gain, check your solvent under flow conditions with the auto-gain function. Do not use higher values than proposed by the system, if not necessary, because of excessive high fluorescence signals.

Use the PMT test to automatically determine the setting.

Changing the Xenon Flash Lamp Frequency

Modes

The lamp flash frequency can be changed into the following modes:

Table 18 Flash Lamp Modes

Positioning	296 Hz (Standard), 560 V	63 mJoule (18.8 W)
	74 Hz (Economy), 560 V	63 mJoule (4.7 W)
Rotation (Multi Ex/Em)	74 Hz (Standard), 950 V	180 mJoule (13.3 W)
	74 Hz (Economy), 560 V	63 mJoule (4.7 W)

Best sensitivity can be expected with "**no economy**", see Figure 46.

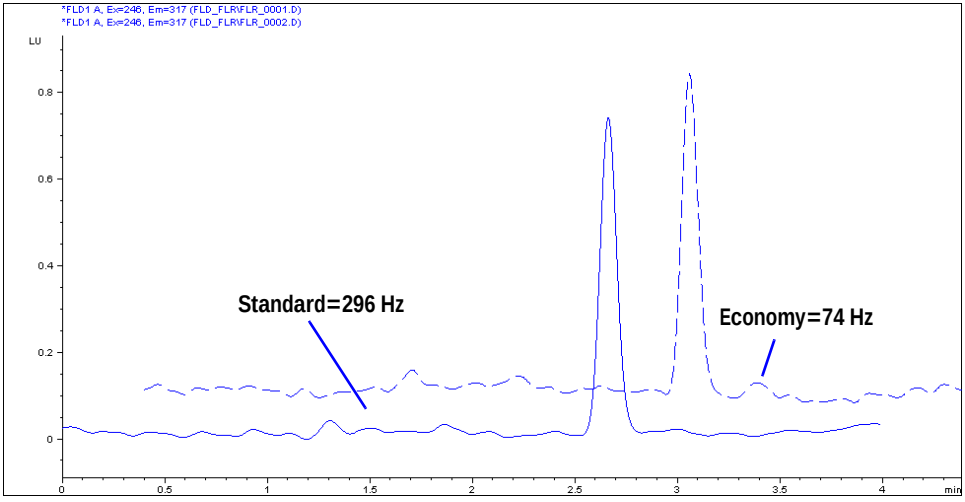


Figure 46 Xenon Flash Lamp Frequency

Lamp life savings

There are three ways to save lamp life:

- switch to “**lamp on during run**” without loss of sensitivity.
- switch to “**economy**” mode with a certain loss of sensitivity.
- a combination of the above.

Selecting the Best Response Time

Data reduction using the RESPONSETIME function will increase your signal-to-noise ratio.

For example, see Figure 47.

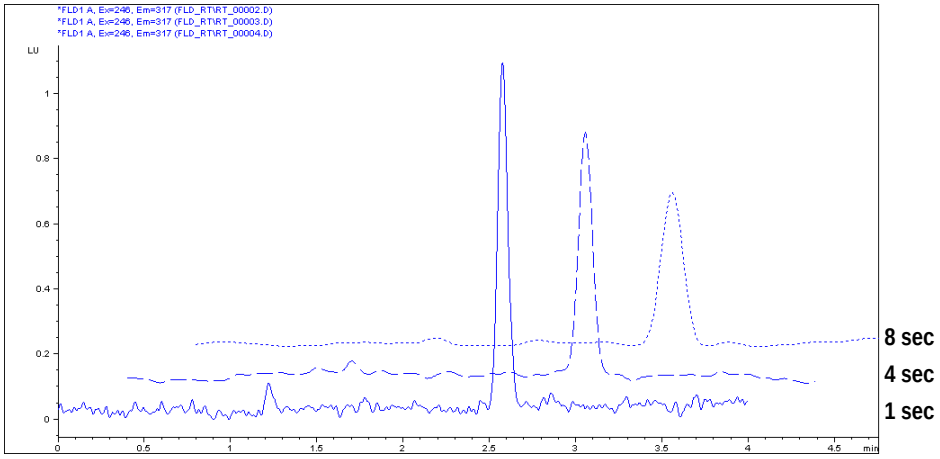


Figure 47 Finding Best Response Time

LC fluorescence detectors typically work with response times of 2 or 4 seconds. The default of the Agilent 1200 Series fluorescence detector is 4 seconds. It is important to know that comparing sensitivity requires using the same response time. A response time of 4 seconds (default) is equivalent to a time constant of 1.8 seconds and appropriate for standard chromatographic conditions.

Figure 48 Separation of Peaks using Responsetime

Reducing Stray Light

Cut-off filters are used to remove stray light and 2nd order or higher stray light by allowing complete transmission above the cut-off and little or no transmission below the cut-off point. They are used between excitation and emission gratings, to prevent any stray excitation light from reaching the photomultiplier tube, when it is measuring emission.

When the emission and excitation wavelengths are close together, the distortion due to scattering severely limits the sensitivity. When the emission wavelength is twice the excitation wavelength the 2nd order light is the limiting factor. To explain the effect of such higher order light, assume the detector is on, but no sample is eluting through the flow cell.

The lamp sends 1 million photons into the flow cell at, for example 280 nm. Scattering on the surface of the flow cell and scattering from the molecules of solvent allow 0.1% of this light to leave the cell through the window at right angles to the incident light. Without a cut-off filter, these remaining 1000 photons will reach the emission grating. 90% will be reflected totally without dispersion onto the photomultiplier. The other 10% disperses at 280 nm (1st order) and at 560 nm (2nd order). To remove this stray light, you need a cut-off filter around 280 nm.

Because of a known set of applications a 295 nm cut-off filter is built-in for undisturbed application up to 560 nm without compromises (see Figure 49 on page 103).

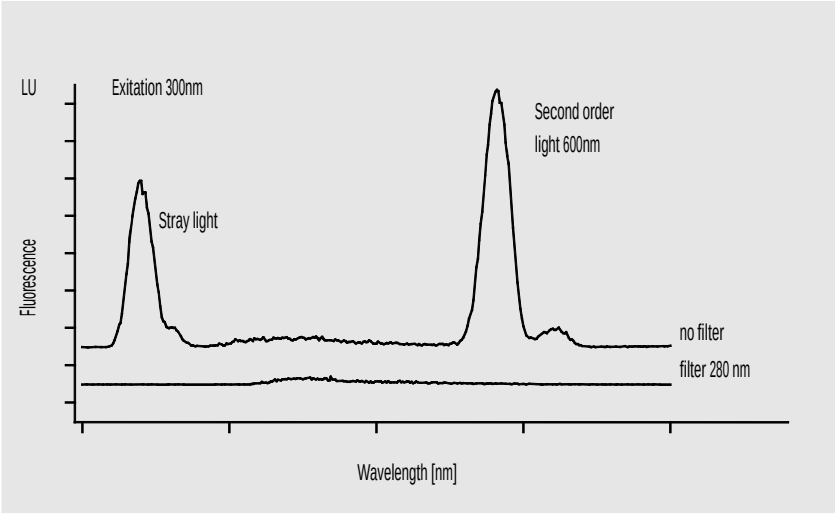


Figure 49 Reducing Stray Light

5 How to optimize the Detector

6
Troubleshooting and Test Functions

Overview of the Detector's Indicators and Test Functions 106
Status Indicators 107
User Interfaces 109
Agilent LC Diagnostic Software 110

This chapter gives an overview about the troubleshooting and diagnostic features and the different user interfaces.

Overview of the Detector's Indicators and Test Functions

Status Indicators

The detector is provided with two status indicators which indicate the operational state (prerun, run, and error states) of the detector. The status indicators provide a quick visual check of the operation of the detector (see page 107).

Error Messages

In the event of an electronic, mechanical or hydraulic failure, the detector generates an error message in the user interface. For each message, a short description of the failure, a list of probable causes of the problem, and a list of suggested actions to fix the problem are provided (see "Error Information" on page 111).

Wavelength Recalibration

Wavelength recalibration is recommended after repair of internal components to ensure correct operation of the detector. The detector uses specific properties of the excitation and emission light characteristics, see "Wavelength Verification and Calibration" on page 189).

Test Functions

A series of test functions are available for troubleshooting and operational verification after exchanging internal components (see "Test Functions" on page 137).

Status Indicators

Two status indicators are located on the front of the detector. The lower left indicates the power supply status, the upper right indicates the detector status.

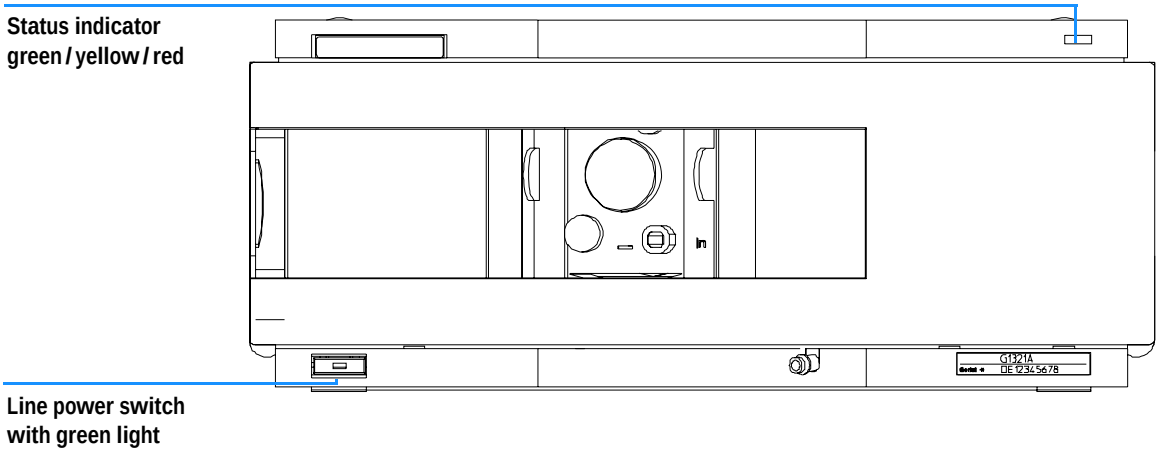


Figure 50 Location of Status Indicators

Power Supply Indicator

The power supply indicator is integrated into the main power switch. When the indicator is illuminated (*green*) the power is ON.

Detector Status Indicator

The detector status indicator indicates one of four possible detector conditions:

- When the status indicator is OFF (and power switch light is on), the detector is in a *prerun* condition, and is ready to begin an analysis.
- A *green* status indicator, indicates the detector is performing an analysis (**run** mode).
- A *yellow* indicator indicates a *not-ready* condition. The detector is in a not-ready state when it is waiting for a specific condition to be reached or completed (for example, immediately after changing a setpoint), or while a self-test procedure is running.
- An *error* condition is indicated when the status indicator is *red*. An error condition indicates the detector has detected an internal problem which affects correct operation of the detector. Usually, an error condition requires attention (e.g. leak, defective internal components). An error condition always interrupts the analysis.

User Interfaces

Depending on the user interface the available tests vary. All test descriptions are based on the Agilent ChemStation as user interface. Some descriptions are only available in the Service Manual.

Table 19 Test Functions avaible vs. User Interface

Test	ChemStation	Instant Pilot G4208A	Control Module G1323B
D/A Converter	No	No	Yes
Test Chromatogram	Yes (C)	No	Yes
Wavelength Calibration	Yes	Yes (M)	Yes
Lamp Intensity	Yes	No	Yes
Dark Current	Yes	No	No

C via command
M section Maintenance
D section Diagnose

NOTE The Agilent Control Module (G1323B) does not do any calculations. So there will be no reports generated with passed/failed information.

Agilent LC Diagnostic Software

The Agilent LC diagnostic software is an application independent tool that provides troubleshooting capabilities for the Agilent 1200 Series modules. It provides for all 1200 Series LC the possibility of a first guided diagnostic for typical HPLC symptoms and a status report stored as Adobe Acrobat pdf or as a printable file to assist users evaluating the instrument state.

At the introduction, following modules will be fully supported by the software, including module tests and calibrations as well as injector steps and maintenance positions.

- Agilent 1200 Series binary pump SL (G1312B)
- Agilent 1200 Series high performance autosampler SL (G1367B)
- Agilent 1200 Series thermostatted column compartment SL (G1316B)
- Agilent 1200 Series diode array detector SL (G1315C)

With further releases of the diagnostic software all Agilent 1200 Series HPLC modules will be fully supported.

This diagnostic software provides tests and diagnostic features that may differ from the descriptions in this manual. For details refer to the help files provided with the diagnostic software.

7
Error Information

What Are Error Messages 112
General Error Messages 113
Detector Error Messages 124

This chapter describes the meaning of detector error messages, and provides information on probable causes and suggested actions how to recover from error conditions.

What Are Error Messages

Error messages are displayed in the user interface when an electronic, mechanical, or hydraulic (flow path) failure occurs which requires attention before the analysis can be continued (for example, repair, or exchange of consumables is necessary). In the event of such a failure, the red status indicator at the front of the detector is switched ON, and an entry is written into the detector logbook.

General Error Messages

General error messages are generic to all Agilent 1200 series HPLC modules.

Time-out

The time-out threshold was exceeded (set in sequence parameter on the Agilent ChemStation or Configure-LC system).

Probable Causes

- The analysis was completed successfully, and the time-out function switched off the pump as requested.
- A not-ready state was present during a sequence or multiple-injection run for a period longer than the time-out threshold.

Suggested Actions

✔ Check the logbook for the occurrence and source of a not-ready condition. Restart the analysis where required.

Shutdown

An external instrument has generated a shut-down signal (through CAN or REMOTE lines).

The detector continually monitors the remote input connectors for status signals. A LOW signal input on pin 4 of the remote connector generates the error message.

Probable Causes

- Leak detected in an external instrument with a remote connection to the system.
- Shut-down in an external instrument with a remote connection to the system.
- The degasser failed to generate sufficient vacuum for solvent degassing.

Suggested Actions

✔ Fix the leak in the external instrument before restarting the pump.

✔ Check external instruments for a shut-down condition.

✔ Check the degasser for an error condition. Refer to the *Reference Manual* for the Agilent 1200 Series vacuum degasser.

Remote Time-out

A not-ready condition is still present on the remote input.

When an analysis is started, the system expects all not-ready conditions (e.g. a not-ready condition during detector balance) to switch to run conditions within one minute of starting the analysis. If a not-ready condition is still present on the remote line after one minute the error message is generated.

Probable Causes

- Not-ready condition in one of the instruments connected to the remote line.
- Defective remote cable.
- Defective components in the instrument showing the not-ready condition.

Suggested Actions

✔ Ensure the instrument showing the not-ready condition is installed correctly, and is set up correctly for analysis.

✔ Exchange the remote cable.

✔ Check the instrument for defects (refer to the instrument's reference documentation).

Synchronization Lost

During an analysis, the internal synchronization or communication between one or more of the modules in the system has failed.

The system processors continually monitor the system configuration. If one or more of the modules is no longer recognized as being connected to the system, the error message is generated.

Probable Causes

- CAN cable disconnected.
- Defective CAN cable.
- Defective main board in another module.

Suggested Actions

✔ Ensure all the CAN cables are connected correctly.

✔ Switch off the system. Restart the system, and determine which module or modules are not recognized by the system.

✔ Ensure all CAN cables are installed correctly.

Leak

A leak was detected in the detector.

The signals from the two temperature sensors (leak sensor and board-mounted temperature-compensation sensor) are used by the leak algorithm to determine whether a leak is present. When a leak occurs, the leak sensor is cooled by the solvent. This changes the resistance of the leak sensor which is sensed by the leak-sensor circuit on the FLM board.

Probable Causes

- Loose fittings.
- Broken capillary.
- Leaking flow cell.

Suggested Actions

✔ Ensure all fittings are tight.

✔ Exchange defective capillaries.

✔ Exchange flow cell.

formation

Detector leak error may be caused by the Agilent 1200 Series sampler. In each sampler injection sequence, step# 2 ejects the mobile phase stored in the metering head during the previous injection. This mobile phase is ejected through the short plastic tube connected to port# 4 of the sampler switching valve. The output of port# 4 is integrated into the sampler's contingency leak drain system, which eventually terminates in the leak pan of the bottom module of the stack - the detector. With normal injection volumes and run times, the output of port# 4 is small, and evaporates right in the sampler leak pan. However, the output of port# 4 is significant, and a substantial volume of ejected mobile phase reaches the detector leak pan.

There are two possible fixes. Select the one which is most convenient.

1 The waste drain plumbing orientation, shown in step 6 on page 59, eliminates the possibility of any leak drainage from above reaching the detector leak pan. The leak drain for the detector can be connected to the detector's leak drain fitting, and taken to waste separately.

2 If it is desired that the system has only one leak drain tube, then it's possible in increase the length of the small plastic tube which is connected to port# 4 of the sampler switching valve. This tube can then be taken to waste separately. The tube which normally serves as the detector cell outlet tube can be used for this purpose.

Leak Sensor Open

The leak sensor in the detector has failed (open circuit).

The current through the leak sensor is dependent on temperature. A leak is detected when solvent cools the leak sensor, causing the leak sensor current to change within defined limits. If the current falls outside the lower limit, the error message is generated.

Probable Causes

- Leak sensor not connected to the FLM board.
- Defective leak sensor.

Suggested Actions

✔ Ensure the leak sensor is connected correctly.

✔ Exchange the leak sensor.

Leak Sensor Short

The leak sensor in the detector has failed (short circuit).

The current through the leak sensor is dependent on temperature. A leak is detected when solvent cools the leak sensor, causing the leak sensor current to change within defined limits. If the current increases above the upper limit, the error message is generated.

Probable Causes

- Defective leak sensor.

Suggested Actions

✔ Exchange the leak sensor.

Compensation Sensor Open

The ambient-compensation sensor (NTC) on the FLM board in the detector has failed (open circuit).

The resistance across the temperature compensation sensor (NTC) on the FLM board is dependent on ambient temperature. The change in resistance is used by the leak circuit to compensate for ambient temperature changes. If the resistance across the sensor increases above the upper limit, the error message is generated.

Probable Causes

- Defective FLM board.

Suggested Actions

✔ Exchange the FLM board.

Compensation Sensor Short

The ambient-compensation sensor (NTC) on the FLM board in the detector has failed (short circuit).

The resistance across the temperature compensation sensor (NTC) on the FLM board is dependent on ambient temperature. The change in resistance is used by the leak circuit to compensate for ambient temperature changes. If the resistance across the sensor falls below the lower limit, the error message is generated.

Probable Causes

- Defective FLF board.

Suggested Actions

✔ Exchange the FLF board.

Fan Failed

The cooling fan in the detector has failed.

The hall sensor on the fan shaft is used by the FLM board to monitor the fan speed. If the fan speed falls below two revolutions/second for more than five seconds, the error message is generated.

Probable Causes

- Fan cable disconnected.
- Defective fan.
- Defective FLM board.

Suggested Actions

✔ Ensure the fan is connected correctly.

✔ Exchange fan.

✔ Exchange the FLM board.

Detector Error Messages

These errors are detector specific.

Lamp Cover Open

The lamp cover in the optical compartment has been removed. The lamp cannot be turned on while this message is on.

Probable Causes

- lamp cover removed.

Suggested Actions

✔ close lamp cover and tighten the screws.

FLF Board not found

The FLF board could not be found by the main board (FLM). This message comes together with some other message generated on the FLF board (e.g. Leak, ...).

Probable Causes

- FLF board not connected to the FLM board.
- defective FLF board.
- defective FLM board.

Suggested Actions

✔ Check the connection between the FLF board and the FLM board.

✔ Exchange the FLF board.

✔ Exchange the FLM board.

ADC Not Calibrated

The analog-to-digital converter located on the FLF board cannot calibrate.

Probable Causes

- defective ADC or other FLF electronics.

Suggested Actions

✔ Exchange the FLF board.

A/D Overflow

This message is not implemented in firmware revision A.03.66 and below.

It indicates an overload situation of the A/D converter (sample signal). The user-interface will show a not-ready condition for the FLD and an info event is written into the logbook. If the message comes up during a run, it includes the time of occurrence and when it disappears.

```
1200 FLD 1 A/D overflow (RT is 0.32 min) 16:33:24 02/11/99
1200 FLD 1 A/D overflow finished (RT is 0.67 min)16:33:46 02/11/99
```

NOTE If this condition is present prior to a run, the not-ready will prevent the system to start the run/sequence.

Probable Causes

- PMT setting to high
- wavelength setting wrong.

Suggested Actions

✔ reduce PMT gain

✔ change wavelength setting.

Flash Lamp Current Overflow

The lamp current of the xenon flash lamp is monitored constantly. If the current gets too high, an error is generated and the lamp is turned OFF.

Probable Causes

- shortage of flash lamp assembly
- shortage of trigger pack assembly.
- defective FLL board.

Suggested Actions

✔ Exchange the flash lamp assembly.

✔ Exchange the FLL board (includes the trigger pack).

Flash Trigger Lost

This message is displayed when the flash trigger is no longer generated.

Probable Causes

- firmware problem
- defective encoder

Suggested Actions

✔ Reboot the detector (power cycle).

✔ Multi Mode Off: exchange the firmware or replace the main board.

✔ Multi Mode Ex: replace EX monochromator assembly.

✔ Multi Mode Em: replace EM monochromator assembly.

Wavelength Calibration Failed

This message may show up during a wavelength calibration.

If the expected deviation is larger than the specified wavelength accuracy, the message "**Wavelength Calibration Failed**" is displayed and the instrument stays in a **Not Ready** condition.

Probable Causes

- Flash lamp not ignited or position not correct.
- Cell position not correct.
- Solvent in the cell not clean or air bubble in the cell.
- monochromator assembly position not correct (after replacement).

Suggested Actions

✔ Check the flash lamp image and position. See also "No Peaks" on page 133.

✔ Check the cell position.

✔ Flush the flow cell.

✔ Reset monochromator settings and re-run the wavelength calibration.

Wavelength Calibration Lost

After exchanging the monochromator assemblies, the calibration factors should be reset to defaults values (a new FLM board comes with default values). In this case "**Wavelength Calibration Lost**" is displayed and the instrument stays in a **Not Ready** condition.

Probable Causes

- Reset of monochromator settings after exchange.
- Replacement of FLM board.

Suggested Actions

✔ Perform a wavelength calibration.

Flow Cell Removed

The detector has an automatic cell recognition system. When the flow cell is removed, the lamp is turned off and a **NOT READY** condition exists. If the flow cell is removed during an analysis, a SHUT DOWN is generated.

Probable Causes

- Flow cell has been removed during analysis.

Suggested Actions

✔ Insert flow cell and turn on the lamp.

No Peaks

If no peaks are shown in the chromatogram, the user-interface shows the module still in "**Ready**". There is no feedback mechanism that checks whether the lamp is ON.

Probable Causes

- defective FLL board / Trigger pack
- defective Xenon flash lamp
- wrong position of monochromator
- FLF board defective

Suggested Actions

✔ Perform a "Lamp Intensity Test" on page 139. If no profile available (very low counts),

✔ replace FLL board / Trigger pack

✔ replace Xenon flash lamp

✔ perform a "Check of Raman Band of Water" on page 156 to check the wavelength calibration.

✔ replace FLF board

Motor Errors

NOTE

Monochromator motor errors may show up during the *initialization* or during *operation* of the detector.

There are individual messages for either the excitation or the emission side.

If an error occurs, do a lamp ignition. This will clear the error and a re-initialization of the motors is performed.

Table 20 Motor Errors

Message	Description	Probable Causes	Suggested Actions
Motor Or Encoder Not Found EX 6705 EM 6706	During initialization of the detector, the excitation and emission monochromator are activated.	encoder cables mixed on FLM board monochromator assembly not connected monochromator or encoder defective monochromator motor power driver defective	check encoder connections to FLM check motor connections to FLF (Ex) and FLM (Em) and encoders to FLM replace monochromator assembly defective FLF board (Ex) or FLM board (Em)
Encoder Index Not Found EX 6707 EM 6708	During initialization of the detector, the excitation- and emission monochromator are activated and the encoder should generate an index.	encoder defective encoder electric defective monochromator defective or missing one phase monochromator motor power driver defective	replace monochromator assembly replace FLM board check for connection to FLF (Ex) and FLM (Em) or replace monochromator assembly defective FLF board (Ex) or FLM board (Em)

Table 20 Motor Errors (continued)

Message	Description	Probable Causes	Suggested Actions
Motor Friction Too High EX 6709 EM 6710	During initialization of the detector, the excitation and emission grating resistance test provides the resistance history of the excitation and the emission grating drives. The number of revolutions after switching off the drives is a measure of friction. The history may show an increasing friction of the drive(s) over a length of time.	friction too high defective monochromator assembly	replace monochromator assembly replace monochromator assembly
Motor Position Not Found EX 6711 EM 6712	When the wavelength is changed the monochromator should move to the new position. The position could not be found.	defective monochromator assembly	reset monochromator settings replace monochromator assembly
Motor Position Lost EX 6713 EM 6714	A mechanical shock to the instrument during operation may cause a movement of the monochromator. The position is lost and the lamp will turn off.	short mechanical shock message appears intermittently without mechanical shock	re-ignite the lamp replace monochromator assembly
Motor Speed Too Low EX 6715 EM 6716	For proper operation the monochromator gratings must run at a certain constant revolution.	revolution too low	replace monochromator assembly
Motor Speed Unstable EX 6717 EM 6718	For proper operation the monochromator gratings must run at a certain constant revolution.	defective monochromator assembly	re-ignite the lamp replace monochromator assembly.
Motor Encoder Index Wrong EX 6717 EM 6718	The actual encoder pattern is checked against a known pattern	encoder was replaced and has a different pattern or no reset of pattern was made encoder lost position completely	reset pattern via user interface and recalibrate replace monochromator assembly

7 Error Information

8
Test Functions

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Wavelength Verification and Calibration 153
Check of Raman Band of Water 156
Using the Agilent ChemStation (OQ/PV) 156
Using the Agilent ChemStation (Manually) 158
Using the Control Module G1323B 161
Wavelength Calibration Procedure 164

This chapter describes the detector's built in test functions.

Diagram of Light Path

The light path is shown in Figure 51.

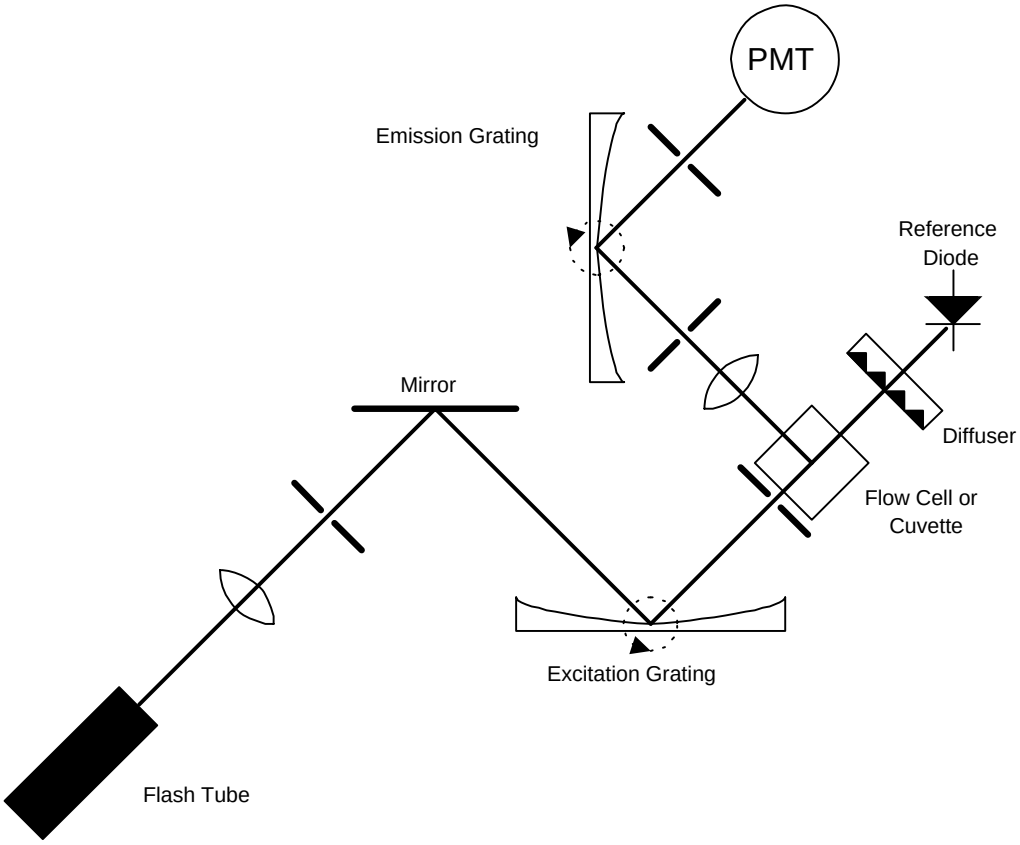


Figure 51 Schematic of the Light Path

Lamp Intensity Test

The intensity test scans an intensity spectrum via the reference diode (200 - 1200 nm in 1 nm steps) and stores it in a diagnosis buffer. The scan is displayed in a graphic window. There is no further evaluation of the test.

Results of this test are stored as lamp history (date code, intensity).

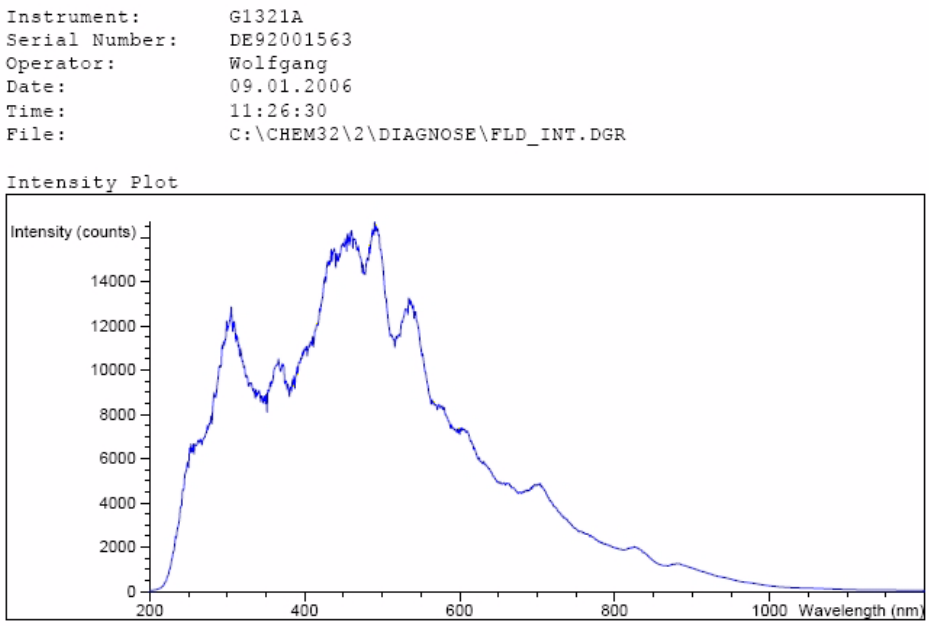


Figure 52 Lamp Intensity Test (Report)

NOTE

The profile can vary from instrument to instrument. It is dependig on the age of the lamp and the content of the flow cell (use fresh water).

UV degradation, especially below 250 nm is significantly higher compared to visible wavelength range. Generally the "**LAMP ON during run**" setting or using "**economy mode**" will increase lamp life by a magnitude.

Lamp Intensity History

Results of the lamp intensity test (if the last one is older than one week) are stored as lamp history (date code, intensity of four different wavelengths, 250 nm, 350 nm, 450 and 600 nm) in a buffer. The data/plot can be retrieved via the diagnostics and provides intensity data over a length of time.

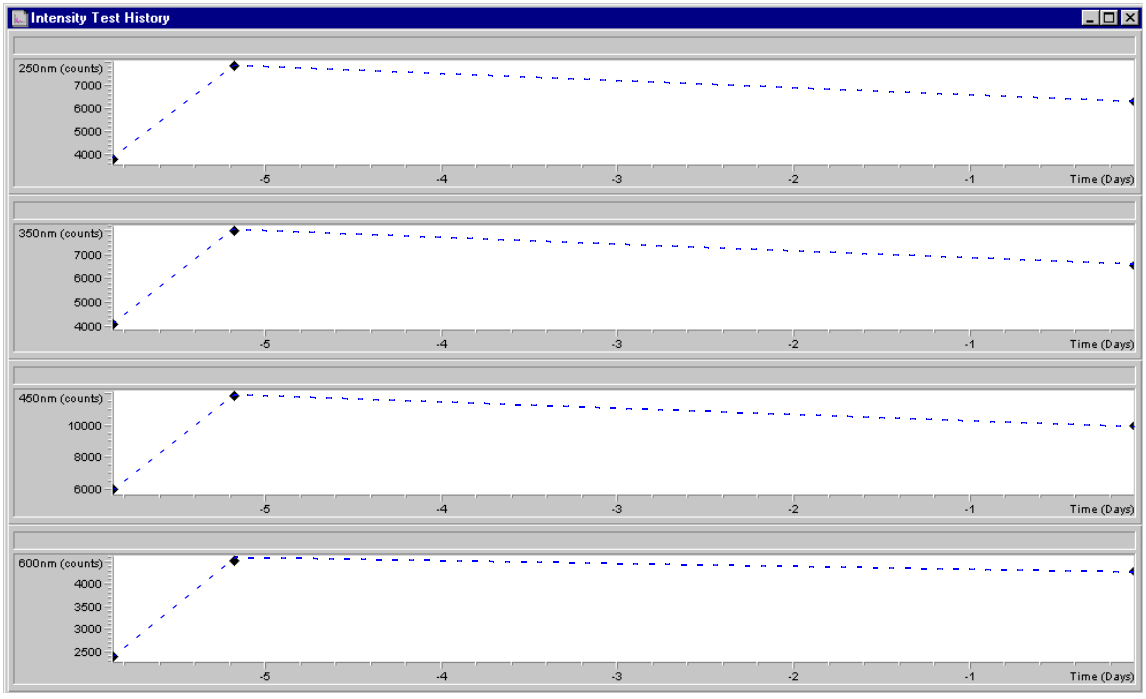


Figure 53 Lamp Intensity History

Dark-Current Test

The dark-current test measures the PMT signal with maximum and minimum gain while the lamp is OFF. It also reads the signal of the reference diode. The resulting values (two via reference diode and two from PMT) are shown in a table and checked against reasonable limits (see below).

```
Instrument:       G1321A
Serial Number:    DE92001563
Operator:         Wolfgang
Date:             09.01.2006
Time:             11:35:34

Dark Current Test Results

                                          Specification    Measured  Result
-------------------------------------------------------------------------
PMT dark current                           < 10000 cts    1227 cts  Passed
Reference diode dark current                < 5000 cts    1023 cts  Passed
```

Figure 54 Dark-Current Test (Report)

Dark-Current Test Evaluation

Test Failed

Probable Causes

- Defective PMT.
- Defective reference diode or A/D converter

Suggested Actions

✔ Exchange the PMT.

✔ Exchange the FLF board

Excitation and Emission Grating Resistance History

This test runs automatically when the instrument is turned on (not accessible as an external test).

It provides the resistance history of the excitation and the emission grating drives. The number of revolutions after switching off the drives is a measure of friction. The history may show an increasing friction of the drive(s) over a length of time.

The history data contains the data/time information and the number of turns. The data/plot can be retrieved via the diagnostics.

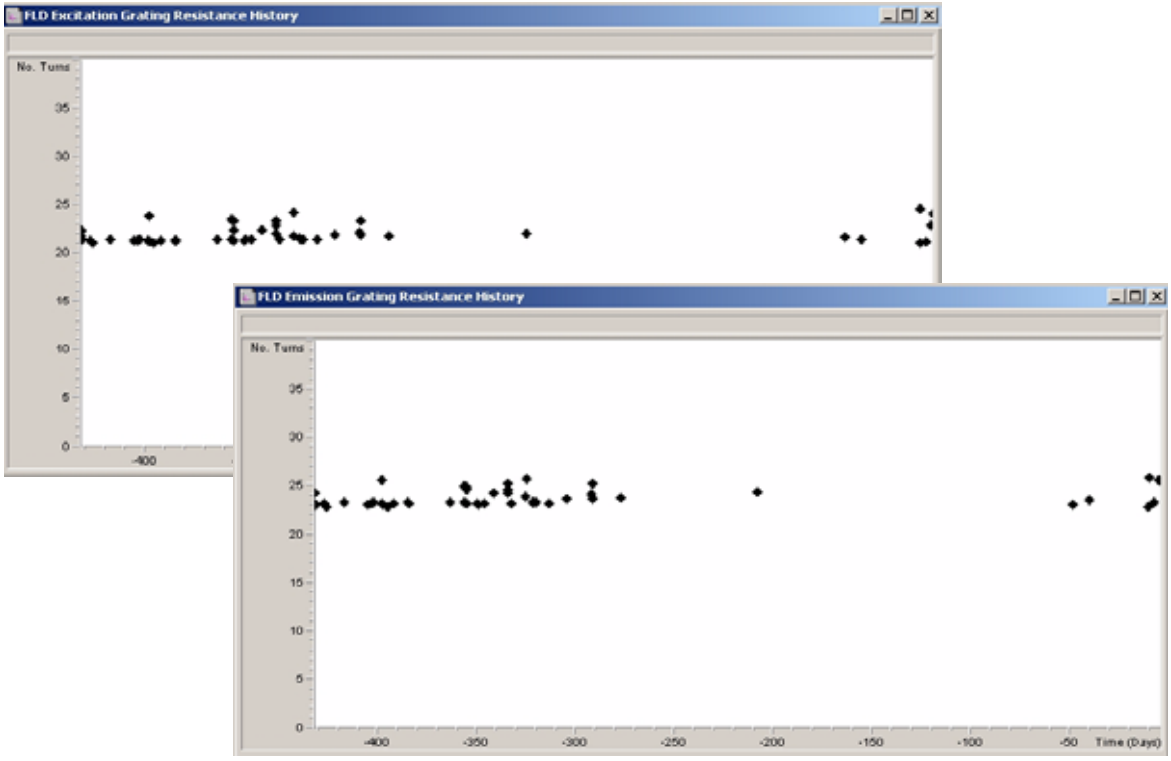


Figure 55 Resistance History

Friction Evaluation

Motor Error

Probable Causes

- Friction too high (< 12 turns)
- Defective monochromator assembly.

Suggested Actions

✔ Exchange the monochromator assembly.

Raman ASTM Signal-to-Noise Test

These tests verify the Raman ASTM signal-to-noise for

- single wavelength (EX=350 nm, EM=397 nm) or
- dual wavelength (EX=350 nm, EM_A=397 nm, EM_B=450 nm)

Table 21 Raman Signal-to-Noise Test Conditions

Duration	approximately 23 minutes
Report Style (Agilent ChemStation)	Performance+Noise
Noise Determination	5 to 20 minutes
Solvent	LC grade water, degassed
Flow rate	0.5 - 1 ml/min
Specification (single wavelength)	>500 (according to settings in Table 22)
Specification (dual wavelength)	>300 (according to settings in Table 23)

Table 22 Settings for Single Wavelength Specifications

Time	EX	EM	PMT	Baseline
0	350	397	12	Free
20.30	350	450	12	Free

Table 23 Settings for Dual Wavelength Specifications (Multi-EM Scan)

Time	EX	EM_A	EM_B	Spectra	From	To	Step	PMT	Baseline	Fit Spectra
00.00	350	397	450	None	280	450	10	12	Free	OFF
20.30	350	450	450	None	280	450	10	12	Free	OFF

Formula for the Raman ASTM S/N value (see Figure 56 for details):

$$RamanASTM = \frac{Height(RamanCurrent) - Height(DarkCurrent)}{ASTMNoise}$$

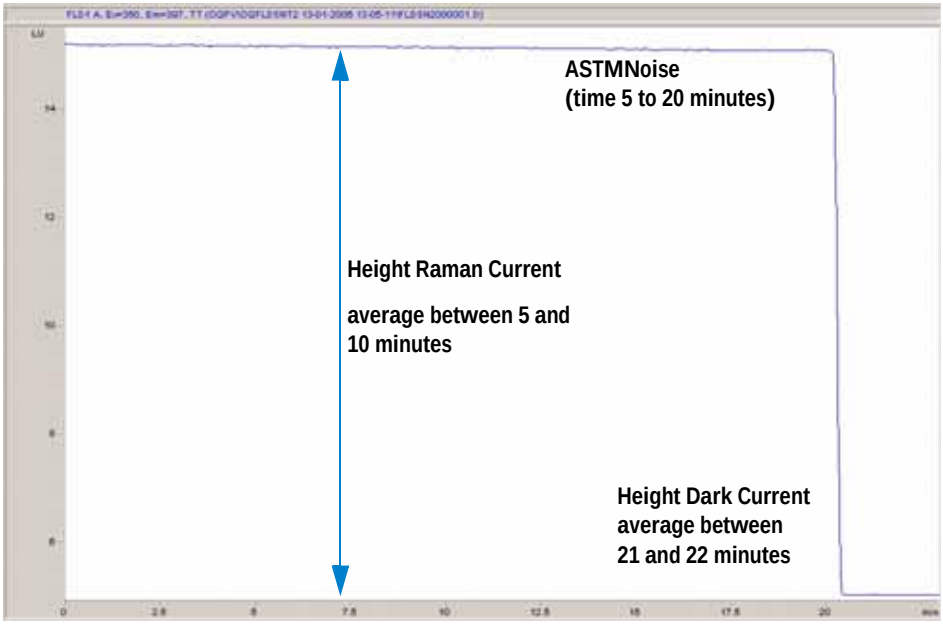


Figure 56 Raman ASTM signal / noise calculation

Procedure using an Agilent ChemStation

1 Set up the HPLC system and the ChemStation.

1 Flush the flow cell with clean bi-distilled water.

2 Turn ON the FLD lamp.

3 Select “**Verification (OQ/PV)**”.

NOTE

Assure that the FLD signal is signal 1 (in case you use an additional detector in the system). Otherwise wrong calculations may be done due to wrong signals.

Single Wavelength Verification

4 Create a **New Instrument Verification**.

5 Select the Signal to Noise/Temp. test.

6 Set the limits to 500.

7 Run the verification.

8 When the run is completed a report is displayed and the status line shows the “**Raman signal/noise ratio =** “ value should be >500.

Dual Wavelength Verification

1 Open **Method and Run Control**.

2 Open method OQFLDSNT.M.

3 Modify the time table according to Table 23 on page 144.

4 Save the method as OQFLDSNT2.M.

5 Open sequence OQFLDSNT.S.

6 Modify the sequence to use a different store location and and to call up method OQFLDSNT2.M.

7 Save the sequence as OQFLDSNT2.S

8 Create a **New Instrument Verification**.

9 Select the Signal to Noise/Temp. test.

10 Select customize sequence and select OQFLDSNT2.S.

11 Set the limits to 300.

12 Run the verification.

13 When the run is completed a report is displayed and the status line shows the "**Raman signal / noise ratio =** " value should be >300.

Interpretation of the Results

If the test shows low Raman values, check for:

- ✔ correctly positioned flow cell,
- ✔ clean flow cell (flush with clean bi-distilled water),
- ✔ no air bubble(s) (check via fluorescence scan or visual check of cell/cuvette),
- ✔ solvent inlet filter (may create air bubbles in flow cell).

Using the Built-in Test Chromatogram

This function is available from the Agilent ChemStation and the Control Module G1323B.

The built-in Test Chromatogram can be used to check the signal path from the detector to the ChemStation and the data analysis or via the analog output to the integrator or data system. An example is shown in Figure 57. The chromatogram is continuously repeated until a stop is executed either by means of a stop time or manually.

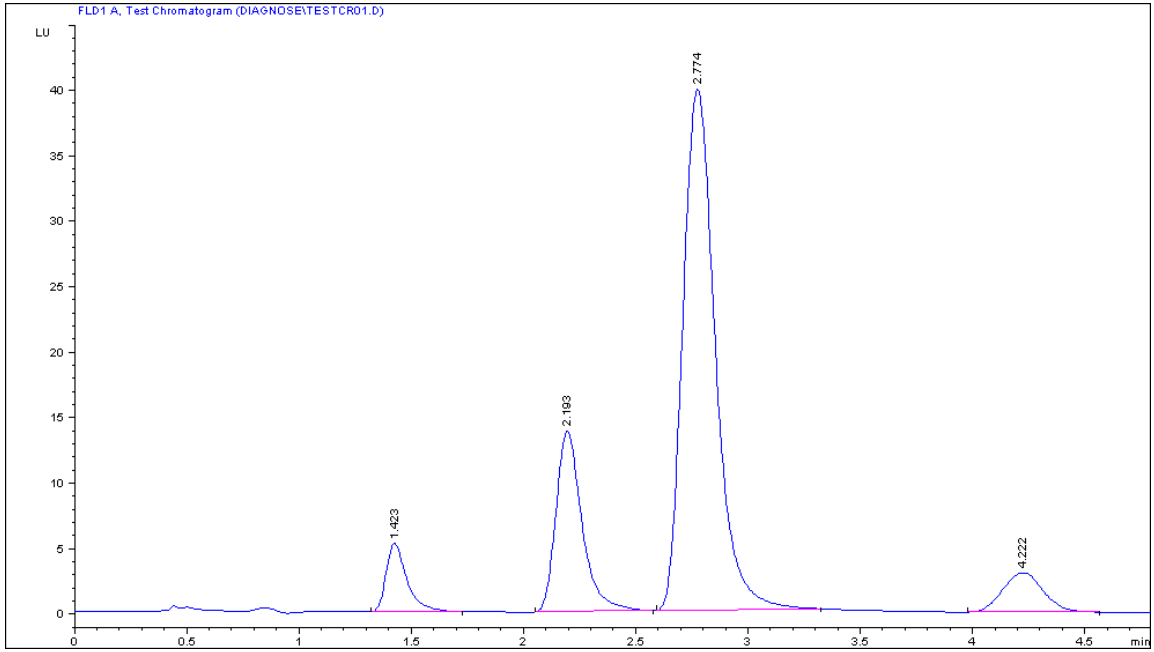


Figure 57 Built-in Test Chromatogram (default settings)

NOTE The peak height is always the same but the area and the retention time depend on the set peakwidth, see examples below.

Table 24

Peakwidth = >0.05 min (response time = 1 s fast)	Stop time = 1.2 minutes
Peakwidth = >0.20 min (response time = 4 s STD)	Stop time = 4.8 minutes

Procedure using the Agilent ChemStation

1 Load the FLD default parameter
   - peakwidth to >0.2 minutes (standard),
   - set stop time to 4.8 minutes.
2 Complete the Sample Information (no injection).
3 Type into the command line (or as pre-run command):

```
PRINT SENDMODULE$(LFLD,"SIMU:DFLT").
```

4 The logbook shows "**Preparing for a simulation**".
5 Start the run.

The results are processed like a regular chromatogram.

NOTE The test chromatogram is switched off automatically at the end of the run.

Procedure using the Control Module G1323B

1 Load the FLD default parameter
   - peakwidth to >0.2 minutes (standard),
   - set stop time to 4.8 minutes.
2 Select **System - Tests - FLD**.
3 Select the function **Enable test chromatogram** and press **Execute**.

   The logbook shows "**Simulated chromatogram enabled**".
4 Enter the **Plot** function and select **Signal A** and/or **Analog x**, set a **time range** of 10 minutes and the **FLD signal** from -1 to 50 LU.

NOTE If an Agilent ChemStation is connected, the next step will start the Agilent ChemStation simultaneously and will overwrite the previous used Data File!

5 Start the run.

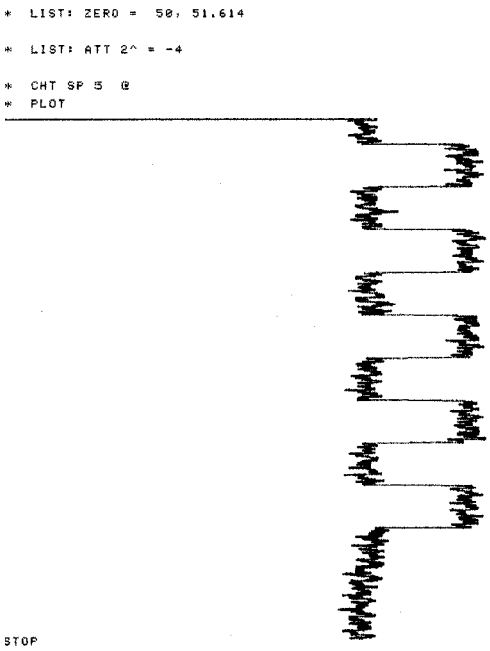
After the run has finished, the plot can be printed to a connected printer by pressing **m** and selecting **Print Plot**.

NOTE The test chromatogram is switched off automatically at the end of the run.

Using the Built-in DAC Test

This function is available from the Control Module G1323B ONLY.

The DAC test provides a test pattern as an analog output. The output voltages (analog 1 and analog 2) should show a constant value corresponding to the ZERO OFFSET value. In addition to the constant voltage is a switched voltage with a duration of 12 seconds and a height of 10 µV, see Figure 58.



Limits:

Noise (pp) < 5 µV

Figure 58 DAC Test (Example)

1 Enable the function (**System - Tests - FLD - Enable DAC Test Pattern**).

2 Start the plot mode (e.g. Agilent 3396 with ATTN -4, ZERO=50, CS=5).

3 Stop the plot and disable the TAC Test (**System - Tests - FLD - Disable DAC Test Pattern**).

4 Evaluate the noise (should be< 5 µV).

Wavelength Verification and Calibration

The wavelength calibration is based on a Glycogen solution, which acts as a strong elastic light scatterer (refer to ASTM Test Method E388-72-1993 "*Spectral Bandwidth and Wavelength Accuracy of Fluorescence Spectrometers*"). The Glycogen solution is introduced into the flow cell and then the built-in wavelength calibration functionality is used.

The algorithm is based on evaluating different grating orders and calculating the wavelength scales of both, excitation and emission monochromator, by applying the fundamental grating equation.

NOTE A complete wavelength calibration is not always required. In most cases a quick wavelength accuracy verification is sufficient enough, see the table below.

Table 25 Reasons for doing a Verification or Calibration

	Verification	WL calibration
interest	X	
GLP compliance	X	
cell change	X	(X)
lamp change	X	(X)
monochromator change		X
main board change		X
optical unit change		X

(X) only required, if deviation is too large.

NOTE Prior to a wavelength calibration, a wavelength accuracy verification should be performed, see "Check of Raman Band of Water" on page 156. If the deviation is more than ±3 nm, the wavelength calibration should be done as described in "Wavelength Calibration Procedure" on page 164.

NOTE The duration of the wavelength calibration is about 15 minutes plus setup time for the calibration sample and system. Depending on the maximum intensity found during this scan, the PMT gain will be changed automatically and requires an additional 1 minute per scan.

Table 26 on page 155 shows the steps performed during the wavelength calibration.

The excitation grating and the emission grating are calibrated using Rayleigh stray light from the flow cell or cuvette measured with the photomultiplier tube.

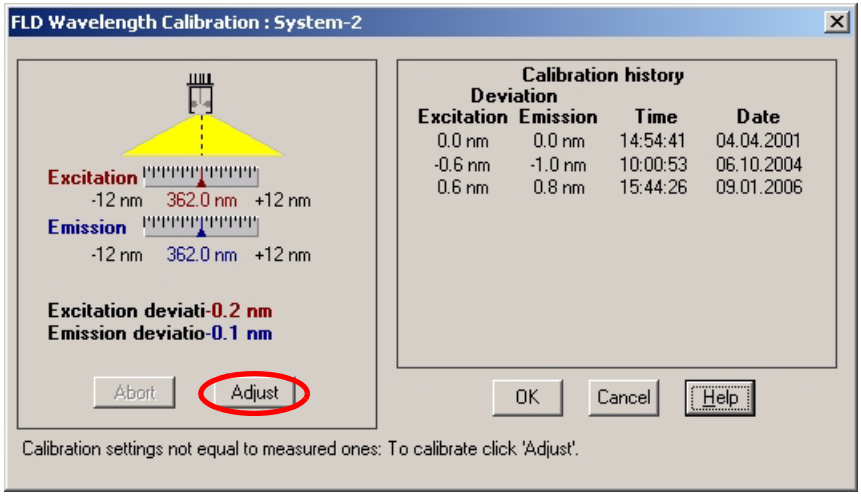


Figure 59 Wavelength Calibration

Table 26 Wavelength Calibration Steps

Step	Description	Duration
1	Preparation	max 30 s
2	Excitation rotation scan, full circle	60 s
3	Excitation rotation scan, high resolution	44 s
4	Excitation position scan, low resolution	55 s variable
5	Excitation position scan, high resolution	260 s variable
6.n	Emission rotation scan, full circle (# of scans depends on the required PMT gain, 1 minute per scan)	61 s variable
6.n	Em rotation scan, full circle" (instrument profile)	9 s
6.n	Em rotation scan, full circle" (instrument profile)	9 s
6.n	Em rotation scan, full circle" (instrument profile)	9 s
6.n	Em rotation scan, full circle" (instrument profile)	9 s
7	Emission rotation scan, high resolution, part I	44 s
8	Emission rotation scan, high resolution, part II	44 s
9	Emission position scan, low resolution	50 s variable
10	Emission position scan, high resolution	250 s variable

NOTE

Variable times means that they could be a little bit longer.

When the lamp is of, the calibration process will stop within the first two steps with “ Wavelength Calibration Failed” , see “Wavelength Calibration Failed” on page 130.

Check of Raman Band of Water

This test is a verification of the wavelength calibration, described in section "Diagram of Light Path" on page 138, using the Raman band of water.

NOTE This test requires the Agilent ChemStation Spectra module or the Control Module G1323B and a running HPLC system.

NOTE If no OQ/PV protocol is available use the procedure "Using the Agilent ChemStation (Manually)" on page 158 or "Using the Control Module G1323B" on page 161.

Using the Agilent ChemStation (OQ/PV)

1 Set up the HPLC system and the ChemStation.

1 Flush the flow cell with clean bi-distilled water.

2 Turn ON the FLD lamp.

3 Select "**Verification (OQ/PV)**".

4 Create a **New Instrument Verification**.

5 Select the "**Excitation Wavelength Accuracy**" and the "**Emission Wavelength Accuracy**" checks (Raman Band of Water).

6 Set the limits to 3 nm on both tests.

7 Run the verification.

8 The FLD will change into the multi-excitation mode with emission wavelength at 397 nm and scan in the range of the expected maximum of 350 nm ±20 nm.

As result, the maxima should be found at 350 nm ±3 nm, see Figure 60 on page 157.

The FLD will change into the multi-emission mode with excitation wavelength at 350 nm and scan in the range of the expected maximum of 397 nm ±20 nm.

As result, the maxima should be found at 397 nm ±3 nm, see Figure 60.

NOTE

If the limits are not met, check for "Interpretation of the Results" on page 147 or perform "Wavelength Calibration Procedure" on page 164.

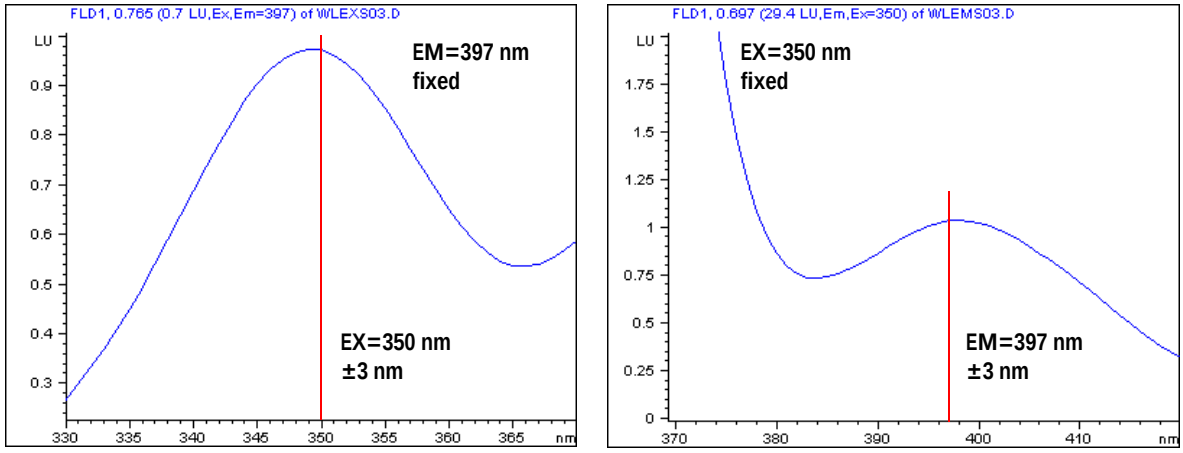


Figure 60 Excitation and Emission Spectrum

Using the Agilent ChemStation (Manually)

1 Create the methods WLEMTEST and WLEXTEST as listed Table 27.

Table 27 Method Settings

Setting	Check of EM WL 397 nm WLEMTEST	Check of EX WL 350 nm WLEXTEST
Peak Width	>0.2 min (4 s, standard)	>0.2 min (4 s, standard)
Fit Spectral Range	OFF	OFF
PMT Gain	10	10
Flash Lamp	ON	ON
Spectrum Range	EM 377 - 417 nm step 1 nm	EX 330 - 370 nm step 1 nm
Store Spectra	All w/o signal	All w/o signal
EX Wavelength	350 nm, ON	350 nm, OFF
EM Wavelength	397 nm, OFF	397 nm, ON
Multi WL Settings	Multi EM	Multi EX

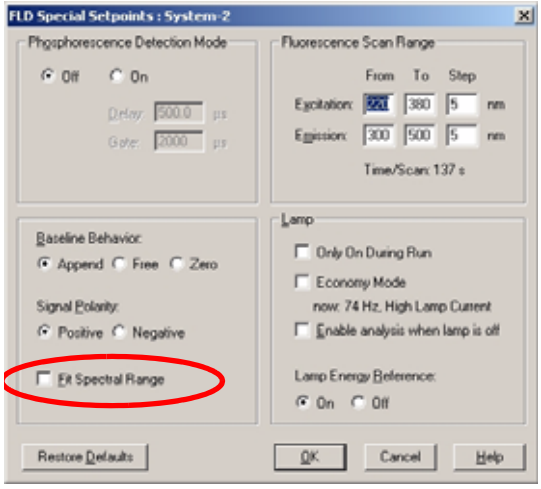


Figure 61 Special Setpoints Settings

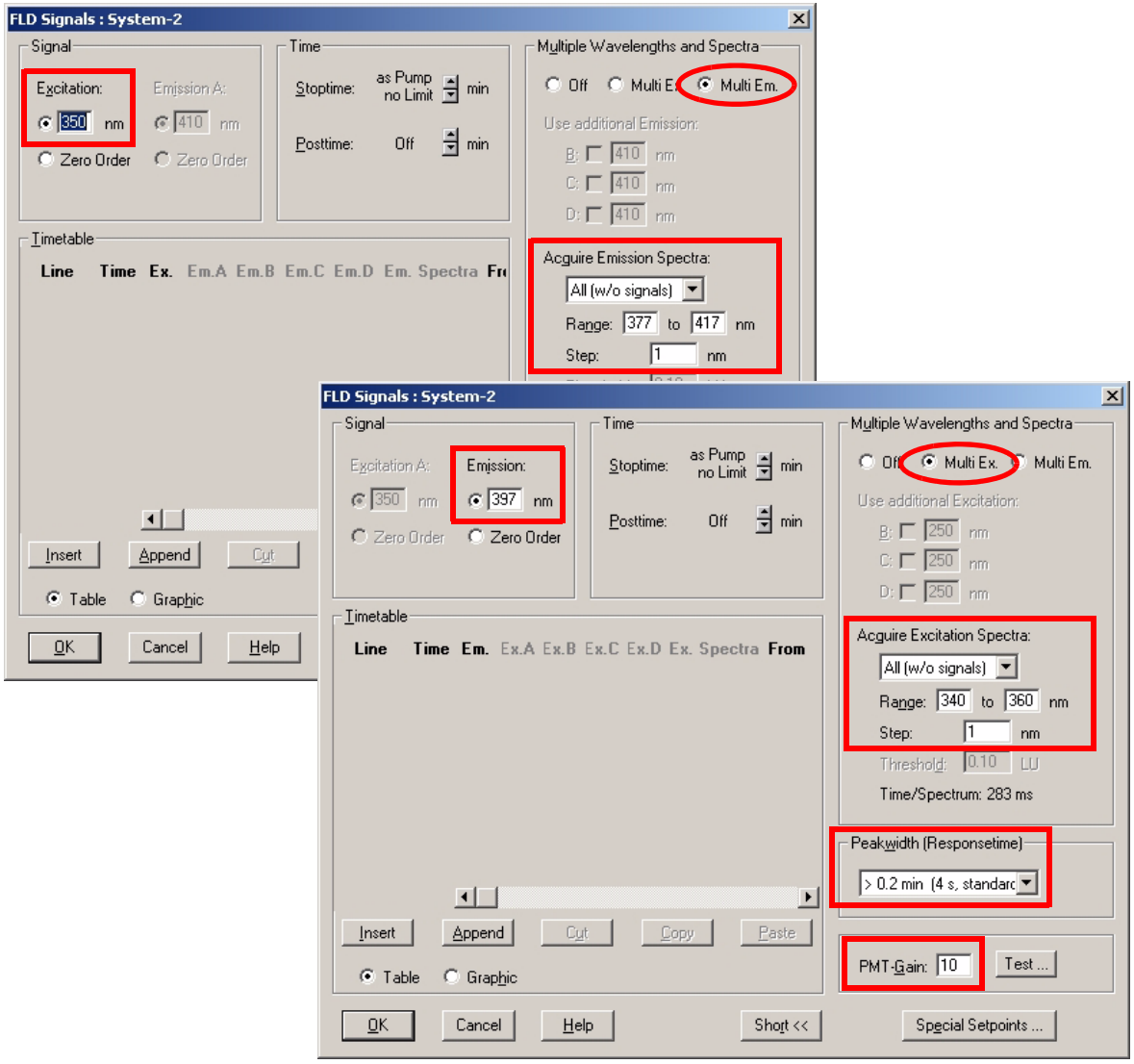


Figure 62 Settings for EM / EX Scan

2 Load the method WLEXTEST. The FLD will change into the multi-emission mode and scan in the range of the expected maximum of 397 nm ±20 nm.

3 Turn the pump ON and flush with water for a few minutes to assure a clean flow cell. Flow rate should be 0.5 to 1 ml/min and the baseline stable.

NOTE

You may remove the flow cell and check for air bubbles. After re-inserting the cell, turn the lamp ON.

4 Open the Online Spectra plot and observe the maximum as shown in Figure 60 on page 157 (left).

5 Load the method WLEMTEST. The FLD will change into the multi-excitation mode and scan in the range of the expected maximum of 350 nm ±20 nm.

6 Open the Online Spectra plot and observe the maximum as shown in Figure 60 on page 157 (right).

Using the Control Module G1323B

1 Create the methods WLEMTEST and WLEXTEST as listed Table 27 on page 158.

2 Copy the methods to your PC card and re-boot the control module.

3 On the **Analysis** screen press **Method** and copy the methods into the module.

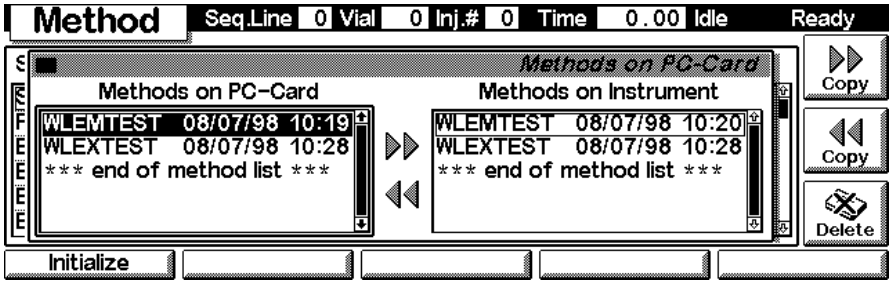


Figure 63 Copying Methods into the FLD

4 Load the method WLEXTEST. The FLD will change into the multi-emission mode and scan in the range of the expected maximum of 397 nm ±20 nm.

5 Turn the pump ON and flush with water for a few minutes to assure a clean flow cell. Flow rate should be 0.5 to 1 ml/min and the baseline stable.

NOTE You may remove the flow cell and check for air bubbles. After re-inserting the cell, turn the lamp ON.

6 From the **Analysis** screen select **Settings - FL Detector**. Then press **Spectrum,** this will open the window below.

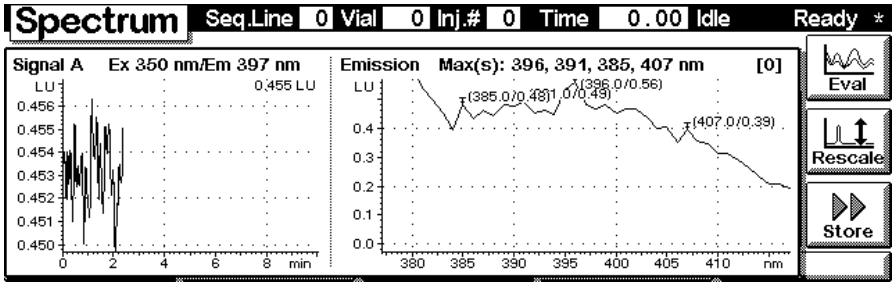


Figure 64 Online Emission Spectra

7 Press **Store** a few times to store a few spectra in a temporary buffer.

8 Press **Eval** to change to the following screen.

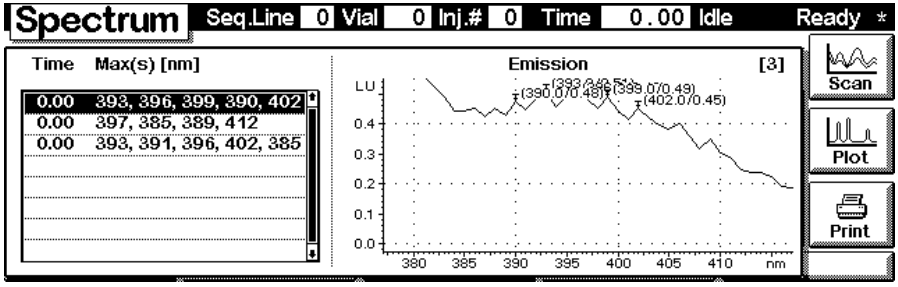


Figure 65 Evaluating Online Emission Spectra

9 You can now select a stored spectra and evaluate/print them. The maximum should be found at 397 nm ±3 nm.

10 Load the method WLEMTEST. The FLD will change into the multi-excitation mode and scan in the range of the expected maximum of 350 nm ±20 nm.

11 Repeat steps step 6 to step 8.

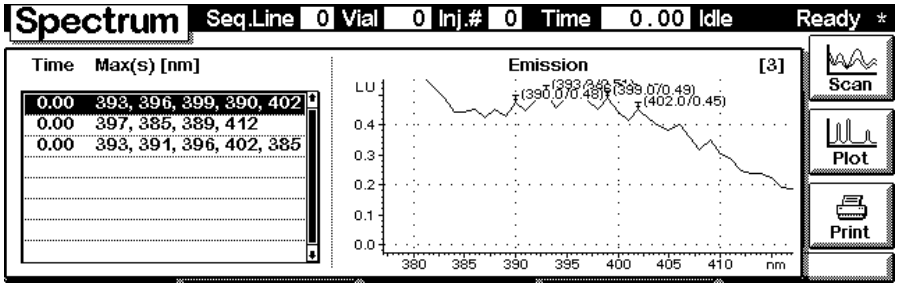


Figure 66 Evaluating Online Excitation Spectra

12 You can now select a stored spectra and evaluate/print them. The maximum should be found at 350 nm ±3 nm.

NOTE The spectra on the control module is a real raw data spectrum. Therefore it looks not that smooth as on the Agilent ChemStation (uses the spline algorithm).

NOTE If the limits are not met, check for "Interpretation of the Results" on page 147 or perform "Wavelength Calibration Procedure" on page 164.

Wavelength Calibration Procedure

When required	If application requires, or see Table 25 on page 153.
Tools required	Laboratory balance
Parts required	Glycogen Calibration Sample, Syringe, needle, sample filter and PEEK fitting from the Accessory Kit, see "Accessory Kit" on page 263

Steps

1 Preparation of the Glycogen Calibration Sample.

2 Preparation of the Flow Cell.

3 Wavelength Calibration.

4 Verification using "Check of Raman Band of Water" on page 156.

Preparation of the Glycogen Calibration Sample

1 To prepare 10 ml of the calibration solution you have to use 10 mg of the Glycogen sample (a tolerance of ±20% is not critical).

2 Fill the prepared amount into a suitable bottle/vial.

3 Fill 10 ml of distilled water into the vial and shake.

4 Wait 5 minutes and shake again. After 10 minutes the solution is ready.

Preparation of the Flow Cell

1 Flush the flow cell with water.
2 Remove the inlet capillary from the flow cell.
3 Take the syringe and fix the needle to the syringe adapter.
4 Suck about 1.0 ml of the calibration sample into the syringe.
5 Keep the syringe in a horizontal position.
6 Remove t#he needle.
7 Add the filter to the syringe and fit the needle to filter.

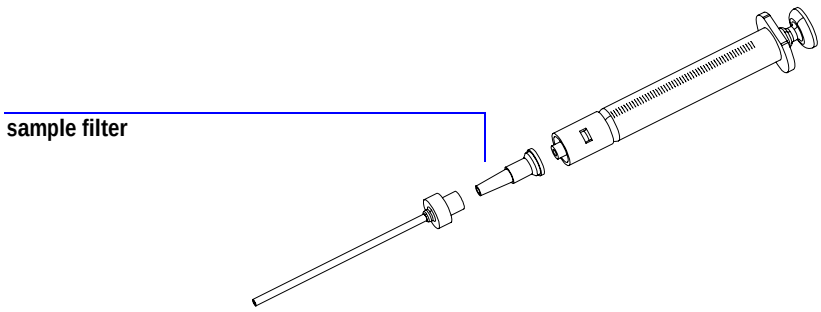


Figure 67 Syringe with Sample Filter

8 Lift the needle tip and carefully eject approximately 0.5 ml to remove air out of the syringe and to flush the needle.
9 Add the PEEK fitting to the needle tip and fix both at the flow cell inlet.

NOTE Do not inject the calibration sample without the sample filter.

10 Slowly inject about 0.2 ml and wait for about 10 seconds to inject another 0.1 ml. This will assure that the cell is filled properly.

Wavelength Calibration

1 From the user interface start the FLD Wavelength Calibration (see Figure 68).
Agilent ChemStation: **Diagnosis - Maintenance - FLD Calibration**
Instant Pilot G4208A: **Maintenance - FLD - Calibration**
Control Module G1323B: **System - Tests - FLD - Calibrate**.

NOTE If the wavelength calibration process fails, refer to "Wavelength Calibration Failed" on page 130.

2 If a deviation is displayed, press **Adjust** and **OK**. The history table will be updated.

NOTE To look at the history table (ChemStation) start a wavelength calibration and abort immediately. No changes are made to the calibration at this time.

NOTE Rinse the flow cell with pure water at a minimum of 1.5 ml/min to get rid of the Glycogen from the cell and the capillaries. When organic solvent is sequentially applied (without rinsing), a blockage of capillaries may occur.

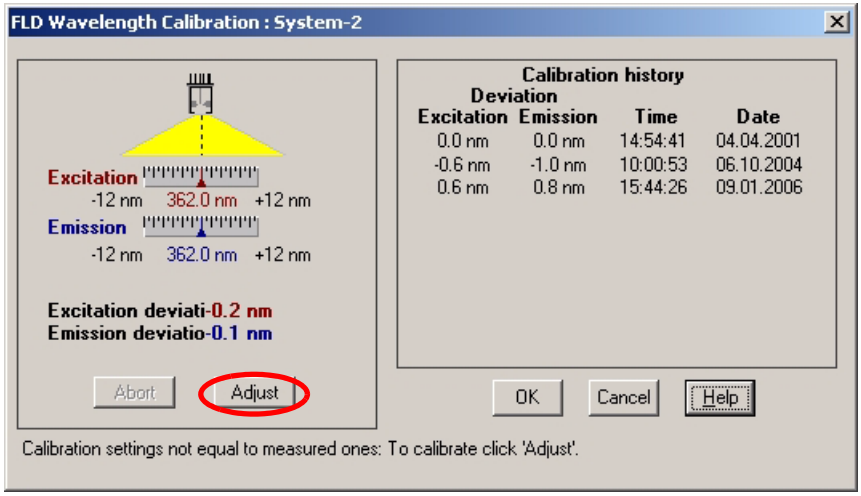


Figure 68 Wavelength Calibration

Verification

1 Refit the capillary to the flow cell.

Follow the procedure "Check of Raman Band of Water" on page 156.

9
Maintenance and Repair

Introduction into Repairing the Fluorescence Detector 170
Warnings and Cautions 171
Cleaning the Detector 173
Using the ESD Strap 174

This chapter provides general information on maintenance and repair of the detector.

Introduction into Repairing the Fluorescence Detector

Simple Repairs

The detector is designed for easy repair. The most frequent repairs such as flow cell change can be done from the front of the detector with the detector in place in the system stack. These repairs are described in “Maintenance” on page 175.

Exchanging Internal Parts

Some repairs may require exchange of defective internal parts. Exchange of these parts (including flash lamp) requires removing the detector from the stack, removing the covers, and disassembling the detector. The security lever at the power input socket prevents the detector cover from being removed when line power is still connected. These repairs are described in “Repairs” on page 195.

Warnings and Cautions

WARNING **To prevent personal injury, the power cable must be removed from the instrument before opening the detector cover. Do not connect the power cable to the detector while the covers are removed.**

WARNING **To prevent personal injury, be careful when getting in contact with sharp metal areas.**

WARNING **When working with solvents please observe appropriate safety procedures (for example, goggles, safety gloves and protective clothing) as described in the material handling and safety data sheet supplied by the solvent vendor, especially when toxic or hazardous solvents are used.**

CAUTION Electronic boards and components are sensitive to electronic discharge (ESD). In order to prevent damage always use an ESD protection when handling electronic boards and components (see “Using the ESD Strap” on page 174).

CAUTION There is a risk of damaging hardware due to overheating when operating the instrument without covers.

WARNING **Eye damage may result from directly viewing the light produced by the Xenon flash lamp used in this product. Always turn the xenon flash lamp off before removing it.**

Cleaning the Detector

The detector case should be kept clean. Cleaning should be done with a soft cloth slightly dampened with water or a solution of water and mild detergent. Do not use an excessively damp cloth allowing liquid to drip into the detector.

WARNING **Do not let liquid drip into the detector. It could cause shock hazard and it could damage the detector.**

Using the ESD Strap

Electronic boards are sensitive to electronic discharge (ESD). In order to prevent damage, always use an ESD strap when handling electronic boards and components.

1 Unwrap the first two folds of the band and wrap the exposed adhesive side firmly around your wrist.

2 Unroll the rest of the band and peel the liner from the copper foil at the opposite end.

3 Attach the copper foil to a convenient and exposed electrical ground.

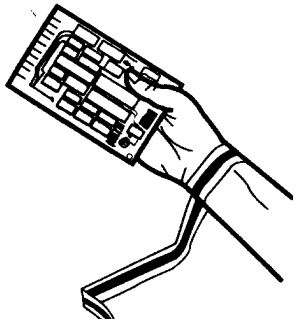


Figure 69 Using the ESD Strap

10 Maintenance

Overview of Maintenance 176
Exchanging a Flow Cell 177
How to use the Cuvette 180
Flow Cell Flushing 181
Correcting Leaks 182
Replacing Leak Handling System Parts 183
Replacing the Interface Board 184
Replacing the Detector's Firmware 185
Tests & Calibrations 186
Lamp Intensity Test 187
Wavelength Verification and Calibration 189
Wavelength Calibration Procedure 191

This chapter describes the maintenance of the detector and the required tests.

Overview of Maintenance

On the following pages repairs are described that can be carried out without opening the main cover.

Table 28 Simple Repairs

Procedure	Typical Frequency	Notes
Flow cell exchange	If application requires a different flow cell type or if defective.	Complete Assembly A wavelength calibration check should be performed after replacement. If the flow cell is removed and inserted, then a quick calibration check is performed. If this fails, you must do a wavelength recalibration, see "Wavelength Verification and Calibration" on page 189.
Flow cell flushing	If flow cell is contaminated	
Leak sensor drying	If leak has occurred.	Check for leaks.
Leak handling System replacement	If broken or corroded.	Check for leaks.

Exchanging a Flow Cell

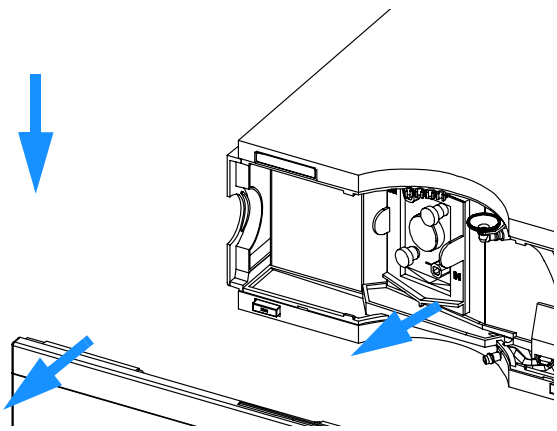
When required	If an application needs a different type of flow cell or the flow cell is defective (leaky).
Tools required	Two 1/4 inch wrenches for capillary connections
Parts required	Standard flow cell, 8 µl, 20 bar, G1321-60005 Cuvette for off-line measurements, 8 µl, 20 bar, G1321-60007, refer to "How to use the Cuvette" on page 180 for more information on usage.

NOTE DO NOT install the inlet capillary to the outlet connection of the flow cell. This will result in poor performance.

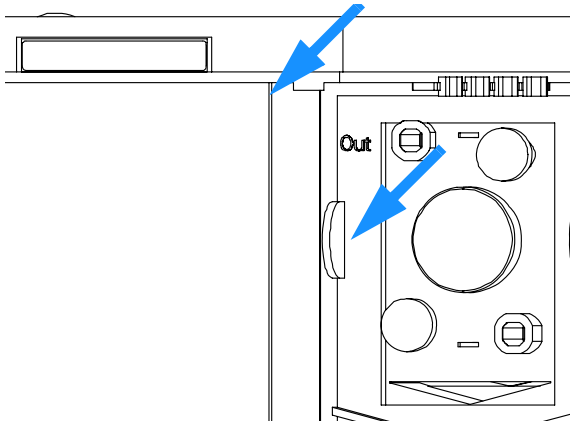
Preparations for this procedure:

- Turn off the flow.

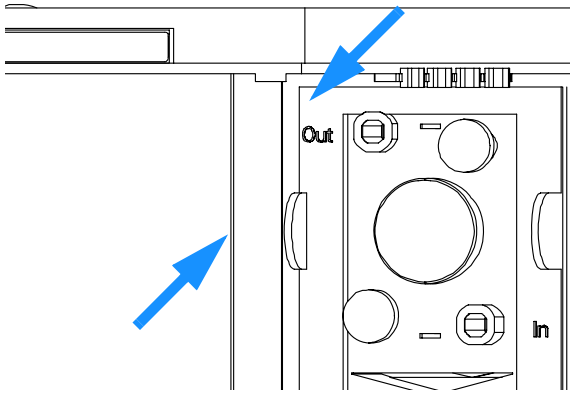
1 Press the release buttons and remove the front cover for access to the flow cell area.



2 Disconnect the capillaries from the flow cell.



3 Unscrew the thumb screws and pull the flow cell out of the compartment.

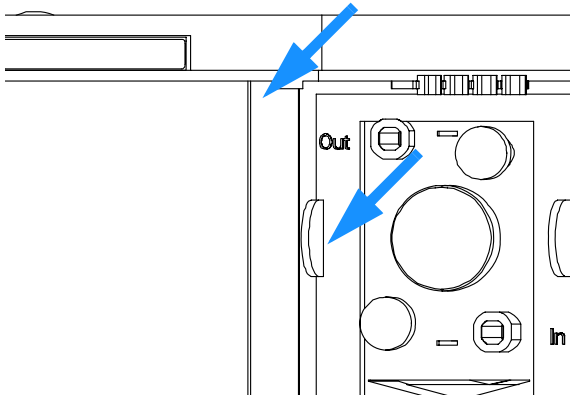


Note:

The label attached to the flow cell provides information on part number, cell volume and maximum pressure. The cell type will be automatically detected.

There are no parts that can be replaced on the flow cell. If defective (leaky) the flow cell has to be replaced completely.

4 Insert the flow cell and tighten the thumb screws. Reconnect the capillaries to the flow cell. DO NOT install the inlet capillary to the outlet connection of the flow cell. This will result in poor performance or damage.



Note:

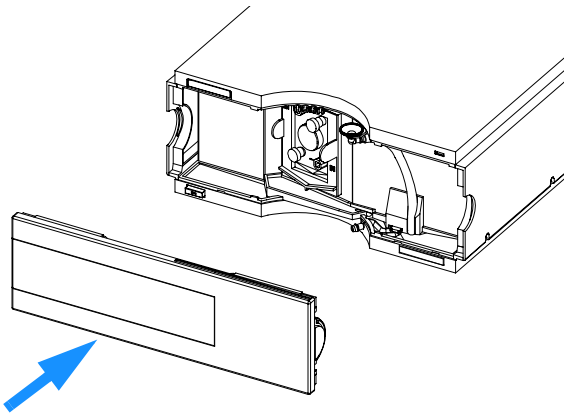
If an additional detector is added to the system, the fluorescence detector should be the last detector in the flow path except for evaporative detectors, like LC-MSD. Otherwise the back pressure generated by the other detector may overload the quartz flow cell and will lead to a defective cell (maximum pressure is 20 bar (2 MPa).

Always use the outlet capillary set supplied with the accessory kit.

Note:

To check for leaks, establish a flow and observe the flow cell (outside of the cell compartment) and all capillary connections.

5 Replace the front cover.



Note:

Perform a wavelength verification to check the correct positioning of the flow cell, as described in chapter "Wavelength Verification and Calibration" on page 189.

How to use the Cuvette

The cuvette is used for off-line measurements (no flow system required) and is basically a standard flow cell with a few changes:

- wide bore capillary connections for easier injections with a syringe
- identification lever for cell auto-recognition system.

1 Install the cuvette instead of the standard flow cell.

2 Connect the waste tubing to the outlet of the cuvette.

3 Use the syringe (see “Cuvette Kit” on page 261) to inject the compound.

4 Setup the parameters for the Fluorescence Scan (under Special Setpoints).

5 Select “Take Fluorescence Scan” on the user-interface to start the off-line measurement.

Flow Cell Flushing

When required	If flow cell is contaminated
Tools required	Glass syringe, adapter
Parts required	Bidistilled water, nitric acid (65%), tubings to waste

NOTE Aqueous solvents in the flow cell can built up algae. Algae do fluoresce. Therefore do not leave aqueous solvents in the flow cell for longer periods. Add a small percentage of organic solvents (e.g. Acetonitrile or Methanol ~5%).

In case the cell is contaminated, follow the procedure below.

Flushing Procedure

1 Flush with bidistilled water.

2 Flush with nitric acid (65%) using a glass syringe.

3 Leave this solution in the cell for about one hour.

4 Flush with bidistilled water.

CAUTION This concentration of nitric acid is dangerous and proper attention to safety should be given. Also the nitric acid flushing procedure is not an infallible remedy for a dirty cell. It is to be used as a last attempt to salvage the cell before cell replacement. Note that the cell is a consumable item.

NOTE Do not exceed the pressure limit of 20 bar (0.2 MPa).

Correcting Leaks

When required	If a leakage has occurred in the flow cell area or at the capillary connections
Tools required	Tissue Two 1/4 inch wrenches for capillary connections
Parts required	None

1 Remove the front cover.

2 Use tissue to dry the leak sensor area and the leak pan.

3 Observe the capillary connections and the flow cell area for leaks and correct, if required.

4 Replace the front cover.

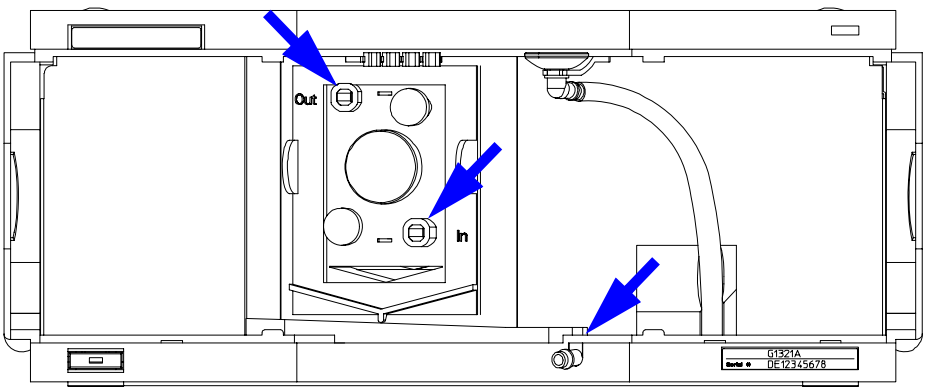


Figure 70 Observing for Leaks

Replacing Leak Handling System Parts

When required	If the parts are corroded or broken
Tools required	None
Parts required	Leak funnel 5061-3356 Leak funnel holder 5041-8389 Leak tubing (120 mm) 0890-1711

1 Remove the front cover.
2 Pull the leak funnel out of the leak funnel holder.
3 Pull out the leak funnel with the tubing.
4 Insert the leak funnel with the tubing in its position.
5 Insert the leak funnel into the leak funnel holder.
6 Replace the front cover.

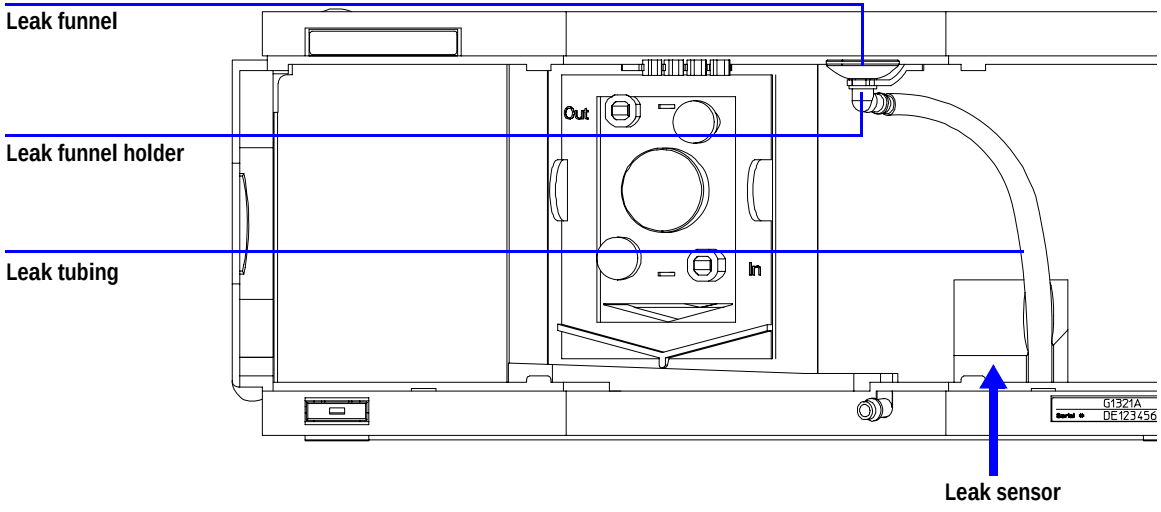


Figure 71 Replacing Leak Handling System Parts

Replacing the Interface Board

When required	For all repairs inside the detector or for installation of the board
Part required	Interface board (BCD) G1351-68701 with external contacts and BCD outputs LAN Communication Interface board G1369A or G1369-60001, see "LAN Communication Interface Board" on page 314
Tools required	None

To replace the interface board unscrew the two screws, remove the board, slide in the new interface board and fix it with the board's screws.

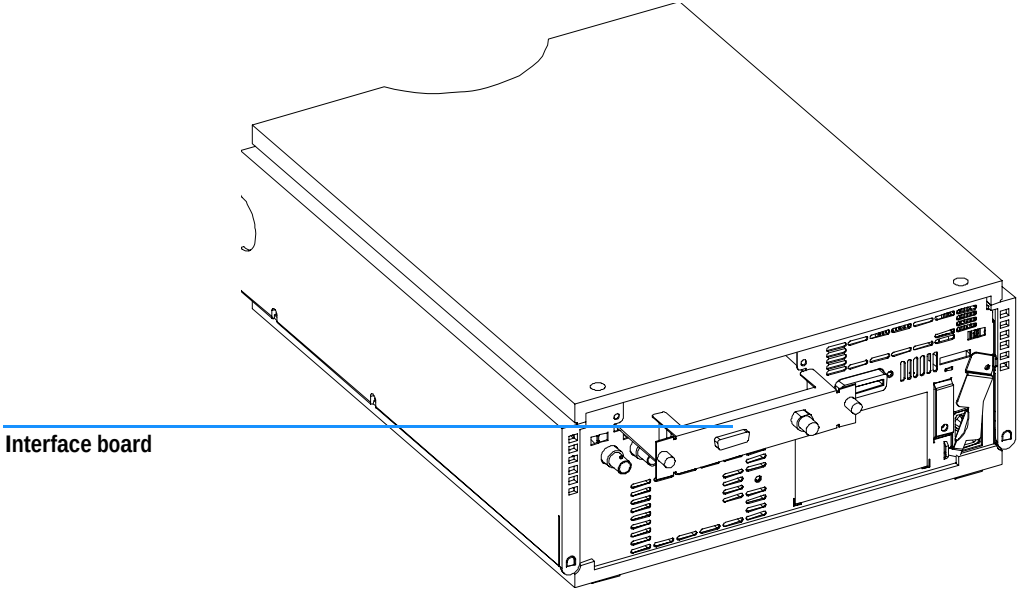


Figure 72 Location of the Interface Board

Replacing the Detector's Firmware

When required	If new version solves problems of currently installed version or after exchange of the detector main board (FLM) the version on board is older than previous installed one.
Tools required	LAN/RS-232 Firmware Update Tool, or Instant Pilot G4208A or Control Module G1323B
Parts required	Firmware, tools and documentation from Agilent web site
Preparations	Read update documentation provided with the Firmware Update Tool.

The installation of *older* firmware might be necessary:

- to keep all systems on the same (validated) revision, or
- if third part control software requires a special version.

To upgrade/downgrade the detector's firmware the following steps have to be performed:

1 Download the module's firmware, the LAN/RS-232 FW Update Tool Version 2.10 or above and the documentation from the Agilent web

http://www.chem.agilent.com/scripts/cag_firmware.asp.

2 Load the firmware into the detector as described in the documentation.

Tests & Calibrations

The following tests are required after maintenance of lamps and flow cells:

- “Lamp Intensity Test” on page 187.
- “Wavelength Verification and Calibration” on page 189.

Lamp Intensity Test

When required	If the flow cell or lamp has been replaced
Tools required	None
Pre-requisites	clean flow cell (flushed)

The intensity test scans an intensity spectrum via the reference diode (200 - 1200 nm in 1 nm steps) and stores it in a diagnosis buffer. The scan is displayed in a graphic window. There is no further evaluation of the test.

Results of this test are stored as lamp history (date code, intensity).

```
Instrument:        G1321A
Serial Number:     DE92001563
Operator:          Wolfgang
Date:              09.01.2006
Time:              11:26:30
File:              C:\CHEM32\2\DIAGNOSE\FLD_INT.DGR
```

Intensity Plot

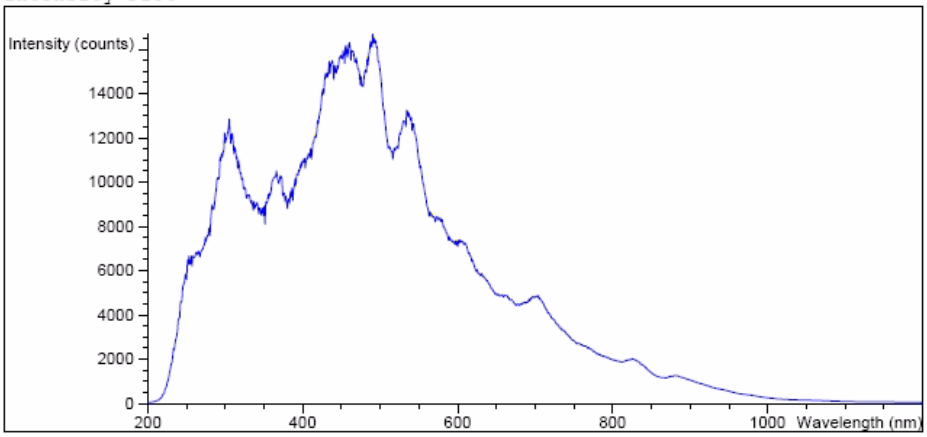


Figure 73 Lamp Intensity Test (Report)

NOTE The profile can vary from instrument to instrument. It is dependig on the age of the lamp and the content of the flow cell (use fresh water).

UV degradation, especially below 250 nm is significantly higher compared to visible wavelength range. Generally the "**LAMP ON during run**" setting or using "**economy mode**" will increase lamp life by a magnitude.

Lamp Intensity History

Results of the lamp intensity test (if the last one is older than one week) are stored as lamp history (date code, intensity of four different wavelengths, 250 nm, 350 nm, 450 and 600 nm) in a buffer. The data/plot can be retrieved via the diagnostics and provides intensity data over a length of time.

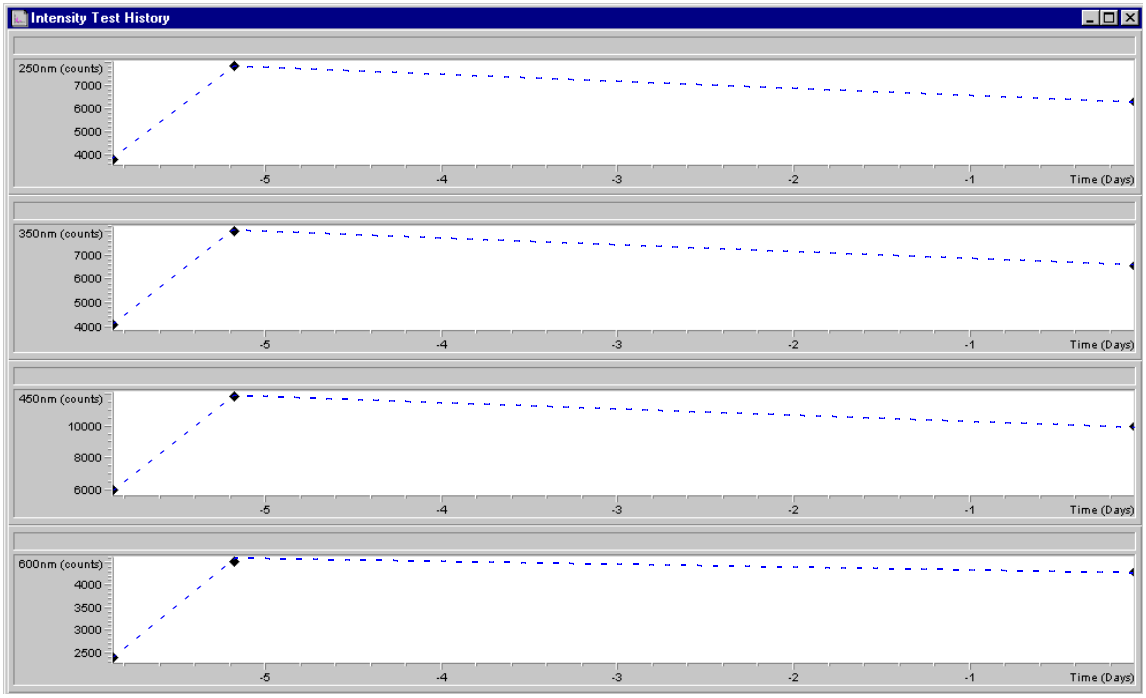


Figure 74 Lamp Intensity History

Wavelength Verification and Calibration

The wavelength calibration is based on a Glycogen solution, which acts as a strong elastic light scatterer (refer to ASTM Test Method E388-72-1993 "*Spectral Bandwidth and Wavelength Accuracy of Fluorescence Spectrometers*"). The Glycogen solution is introduced into the flow cell and then the built-in wavelength calibration functionality is used.

The algorithm is based on evaluating different grating orders and calculating the wavelength scales of both, excitation and emission monochromator, by applying the fundamental grating equation.

NOTE

The duration of the wavelength calibration is about 15 minutes plus setup time for the calibration sample and system. Depending on the maximum intensity found during this scan, the PMT gain will be changed automatically and requires an additional 1 minute per scan.

The excitation grating and the emission grating are calibrated using Rayleigh stray light from the flow cell or cuvette measured with the photomultiplier tube.

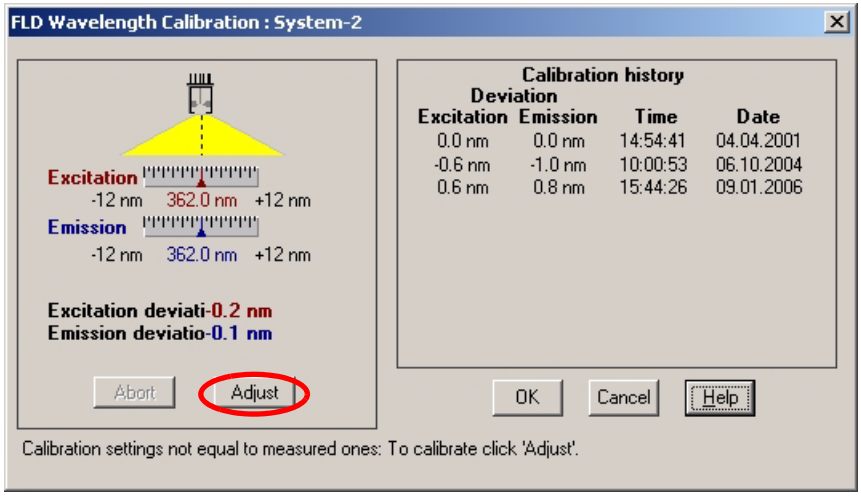


Figure 75 Wavelength Calibration

NOTE When the lamp is of, the calibration process will stop within the first two steps with “Wavelength Calibration Failed”.

Wavelength Calibration Procedure

When required	If application requires, or after replacement of flow cell or lamp.
Tools required	Laboratory balance
Parts required	Glycogen Calibration Sample, Syringe, needle, sample filter and PEEK fitting from the Accessory Kit, see "Accessory Kit" on page 263

Steps

1 Preparation of the Glycogen Calibration Sample.

2 Preparation of the Flow Cell.

3 Wavelength Calibration.

4 Verification using "Check of Raman Band of Water" on page 156.

Preparation of the Glycogen Calibration Sample

1 To prepare 10 ml of the calibration solution you have to use 10 mg of the Glycogen sample (a tolerance of ±20% is not critical).

2 Fill the prepared amount into a suitable bottle/vial.

3 Fill 10 ml of distilled water into the vial and shake.

4 Wait 5 minutes and shake again. After 10 minutes the solution is ready.

Preparation of the Flow Cell

1 Flush the flow cell with water.
2 Remove the inlet capillary from the flow cell.
3 Take the syringe and fix the needle to the syringe adapter.
4 Suck about 1.0 ml of the calibration sample into the syringe.
5 Keep the syringe in a horizontal position.
6 Remove t#he needle.
7 Add the filter to the syringe and fit the needle to filter.

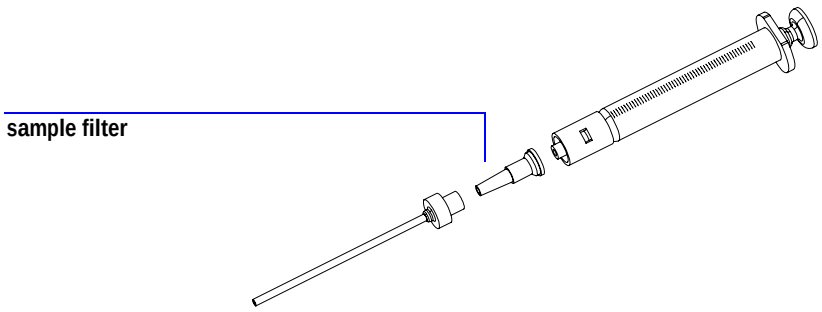


Figure 76 Syringe with Sample Filter

8 Lift the needle tip and carefully eject approximately 0.5 ml to remove air out of the syringe and to flush the needle.
9 Add the PEEK fitting to the needle tip and fix both at the flow cell inlet.

NOTE Do not inject the calibration sample without the sample filter.

10 Slowly inject about 0.2 ml and wait for about 10 seconds to inject another 0.1 ml. This will assure that the cell is filled properly.

Wavelength Calibration

1 From the user interface start the FLD Wavelength Calibration.
Agilent ChemStation: **Diagnosis - Maintenance - FLD Calibration**
Instant Pilot G4208A: **Maintenance - FLD - Calibration**
Control Module G1323B: **System - Tests - FLD - Calibrate**.

NOTE If the wavelength calibration process fails, refer to "Wavelength Calibration Failed" on page 130.

2 If a deviation is displayed, press **Adjust** and **OK**. The history table will be updated.

NOTE To look at the history table (ChemStation) start a wavelength calibration and abort immediately. No changes are made to the calibration at this time.

NOTE Rinse the flow cell with pure water at a minimum of 1.5 ml/min to get rid of the Glycogen from the cell and the capillaries. When organic solvent is sequentially applied (without rinsing), a blockage of capillaries may occur.

11 Repairs

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Assembling the Main Cover 256

This chapter describes the repairs of the detector.

Cautions and Warnings

WARNING **The following procedures require opening the main cover of the detector. Always ensure the detector is disconnected from the line power when the main cover is removed. The security lever at the power input socket prevents the detector cover from being removed when line power is still connected.**

WARNING **To disconnect the detector from line, unplug the power cord. The power supply still uses some power, even if the switch on the front panel is turned off.**

WARNING **When working with solvents please observe appropriate safety procedures (for example, goggles, safety gloves and protective clothing) as described in the material handling and safety data sheet supplied by the solvent vendor, especially when toxic or hazardous solvents are used.**

NOTE Always operate the detector with the top covers in place.

CAUTION There will be a risk of damaging hardware due to overheating when operating the instrument without covers.

CAUTION Electronic boards and components are sensitive to electronic discharge (ESD). In order to prevent damage always use an ESD protection when handling electronic boards and components, see “Using the ESD Strap” on page 174.

WARNING **Eye damage may result from directly viewing the light produced by the xenon flash lamp used in this product.**

Removing the Covers

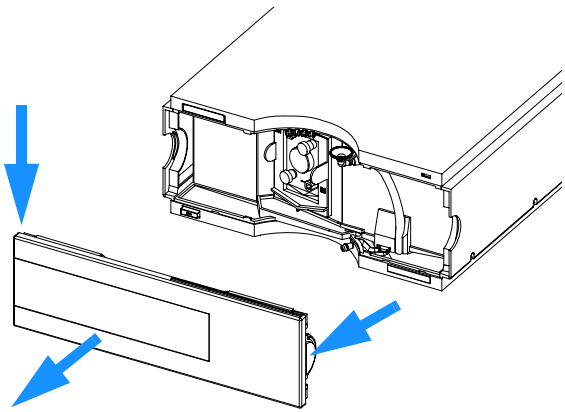
When required	For all repairs inside the detector
Tools required	Screwdriver POZI 1 PT3
Parts required	Depends on the work inside and the following procedures

CAUTION There will be a risk of damaging hardware due to overheating when operating the instrument without covers.

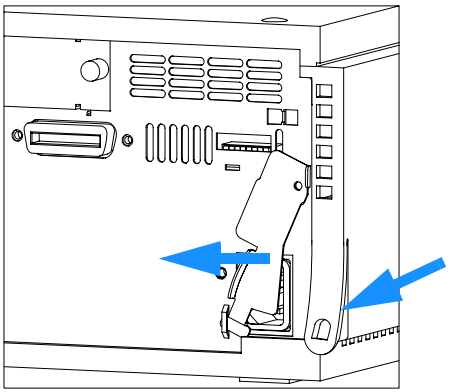
Preparations for this procedure:

- Turn OFF the detector.
- Disconnect the power cable.
- Remove the flow cell.
- Remove detector from stack and place it on the workbench.

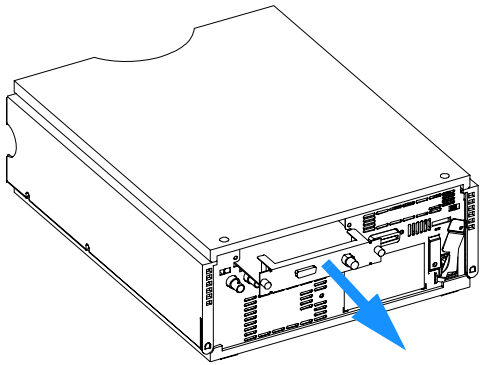
1 Press the release buttons and remove the front cover. Unclip and remove the waste funnel assemly.



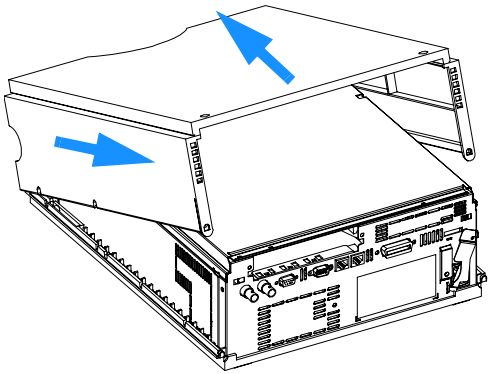
2 Install the ESD strap. Move the power lock across the power inlet and lift the clips on the rear of the cover.



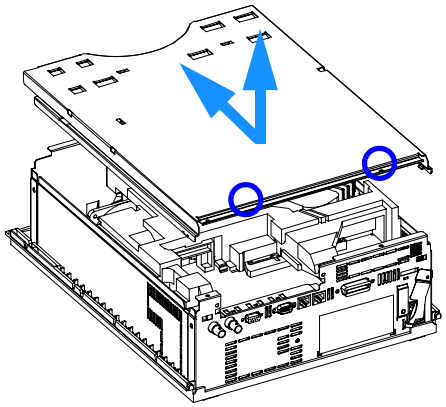
3 If installed, unscrew and remove the interface board. Place the board on the ESD kit.



4 Lift the cover up and slide it towards the rear.



5 Unscrew the screws at the rear of the top plate. Slide the plate towards the front and remove it.



Warning:

Do not connect a power plug to the detector module after removing the top covers.

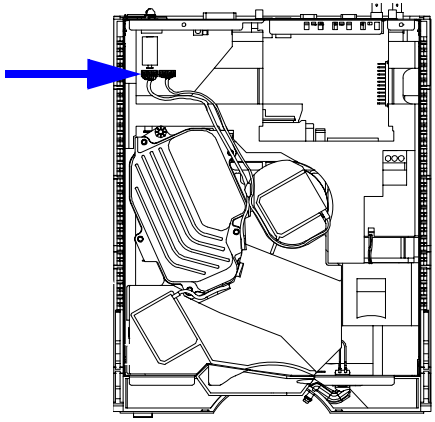
Removing the Rear Foam

When required	For all repairs inside the detector
Parts required	Depends on the work inside and the following procedures

Preparations for this procedure:

- Start with “Removing the Covers” on page 198.

1 Using a pliers disconnect the cables from the main board.



2 Carefully remove the rear foam.

Exchanging the Lamp

When required	Lamp: if noise or intensity exceeds limits or lamp does not ignite
Tools required	Screwdriver POZI 1 PT3 Hexagonal screwdriver 2.5 mm, 100 mm long Hexagonal screwdriver 4mm, 100 mm long
Parts required	Flash lamp 2140-0600

WARNING **Wait until the lamp has cooled down before touching the lamp and / or triggerpack assembly.**

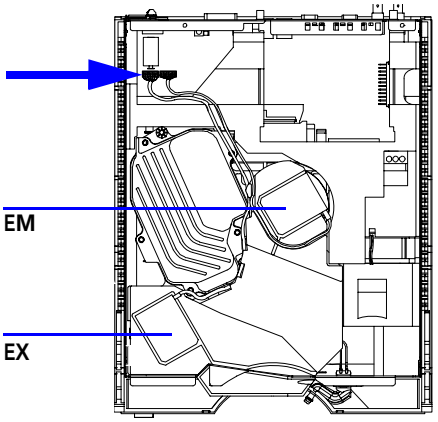
The instrument should not be operated without Xenon flash lamp. Otherwise the Triggerpack electronics will be damaged.

NOTE When loosening or removing screws take care that they do not fall into the instrument. This may result in a complete removal of other assemblies.

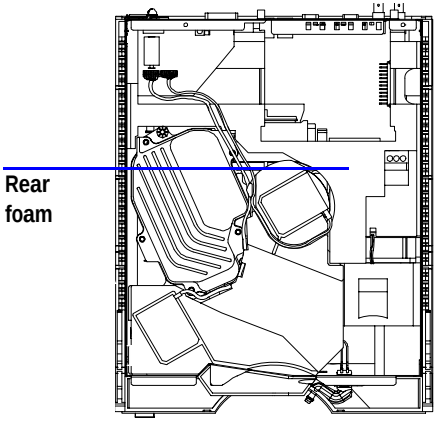
Preparations for this procedure:

- Turn OFF the detector.
- Disconnect the power cable.
- Remove the flow cell or disconnect the tubings from the flow cell.
- Remove detector from stack and place it on the workbench.
- Remove the covers as described in "Removing the Covers" on page 198.

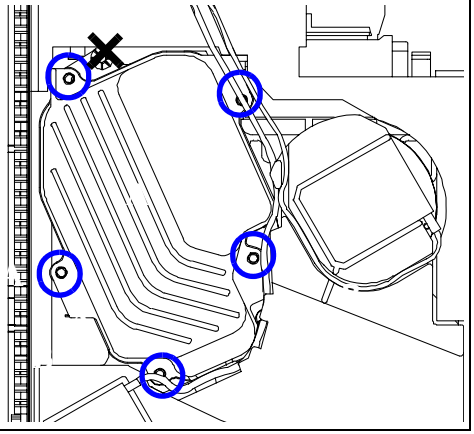
1 Disconnect the emission and the excitation cables and from the detector main board.



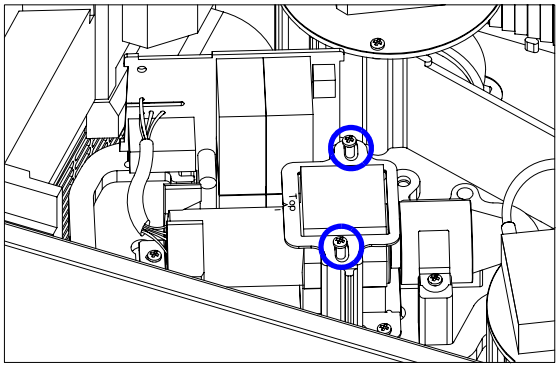
2 Carefully remove the rear foam.



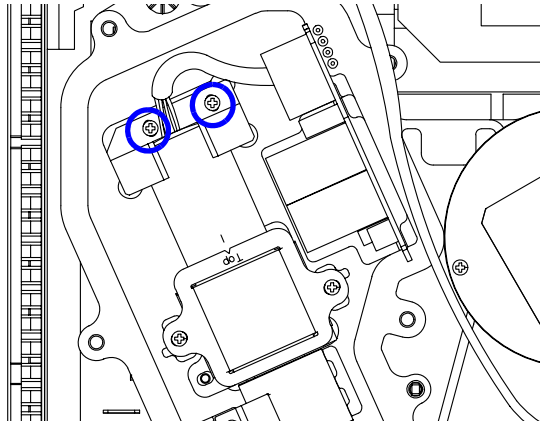
3 Using the 4 mm hexagonal key, unscrew the five screws, remove the cover and place it on the bench without turning it around. **DO NOT unscrew the grounding screw (marked with X)**.



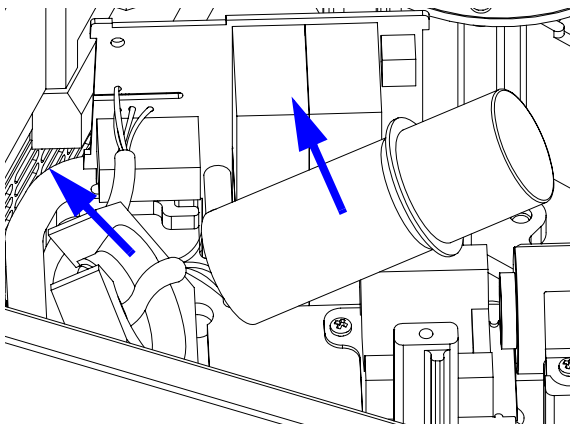
4 Using the 2.5 mm hexagonal key, unscrew the two screws and remove the brass pad together with its holder.



5 Loosen the screws of the trigger pack holder a few turns.



6 Tip the holder to release the lamp and trigger pack. Slide the cables of the trigger pack through the holder.



WARNING:

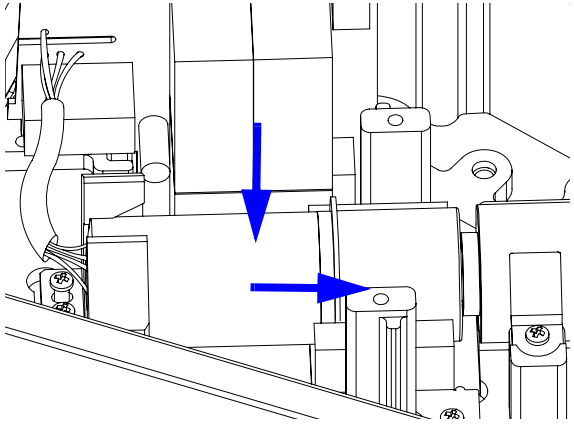
While pulling off the lamp be careful that you do not scrape your skin on the metal plates.

Do not remove the bottom pad. Otherwise the lamp will loose its correct position during reinstallation.

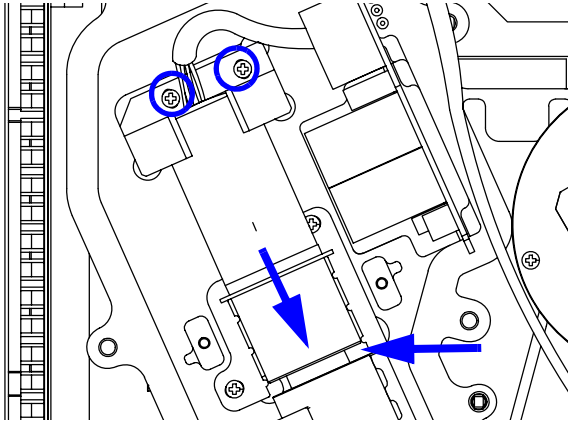
7 Remove the lamp carefully from the trigger pack.

8 Carfully insert the new lamp completely into the trigger pack and slide the cables through the holder.

9 Place the lamp on the bottom pad.



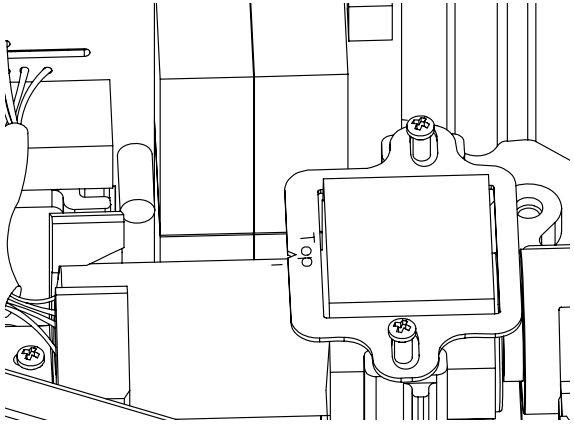
10 Refit the trigger pack holder by moving the lamp towards the condenser. Leave a distance of about 0.5 mm between each other. Refit the screws.



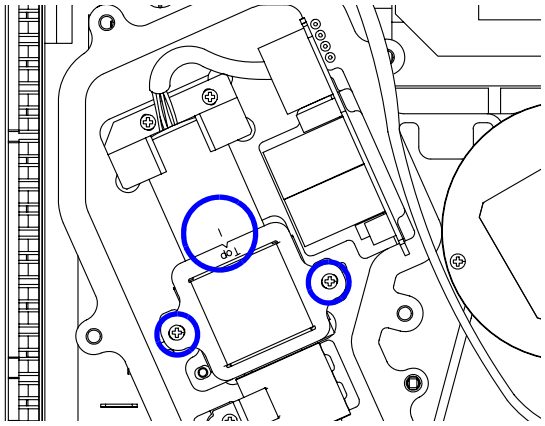
11 Place the pad with the holder on the stand-offs. The "TOP" sign should be directed towards the trigger pack. Refit the screws.

Note:

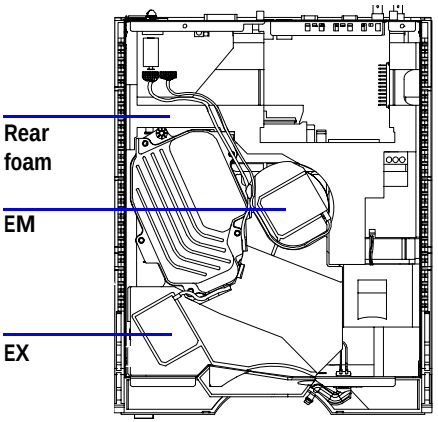
During the next step no horizontal movement should be made to the pad.



12 While pressing the top pad down onto the stand-offs, tighten the screws. Turn the trigger pack such as its marker stays in line with the “TOP” sign of the brass holder.



13 Replace the lamp housing cover and fix the screws. Carefully insert the rear foam. Reconnect the monochromator cables to the main board. Left is excitation, right is emission.



Next Steps:

- Replace the covers as described in “Replacing the Foam and the Top Cover” on page 149.
- Re-install the detector in the stack.
- Enter the replacement of the lamp in the maintenance logbook (this will reset the lamp counter).
- Turn the lamp ON.
- Perform a wavelength verification to check the correct positioning of the replaced assemblies, as described in chapter “Diagram of Light Path” on page 138.

Exchanging the EX-condenser

When required	Condenser: if contaminated
Tools required	Screwdriver POZI 1 PT3 Hexagonal screwdriver 2.5 mm, 100 mm long Hexagonal screwdriver 4 mm, 100 mm long
Parts required	EX condenser Assembly 1000-1136

WARNING **Wait until the lamp has cooled down before touching the lamp and / or triggerpack assembly.**

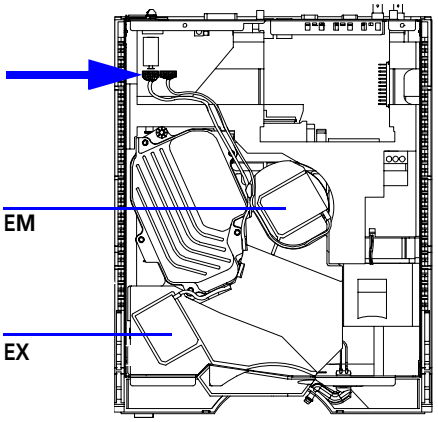
The instrument should not be operated without Xenon flash lamp. Otherwise the Triggerpack electronics will be damaged.

NOTE When loosening or removing screws take care that they do not fall into the instrument. This may result in a complete removal of other assemblies.

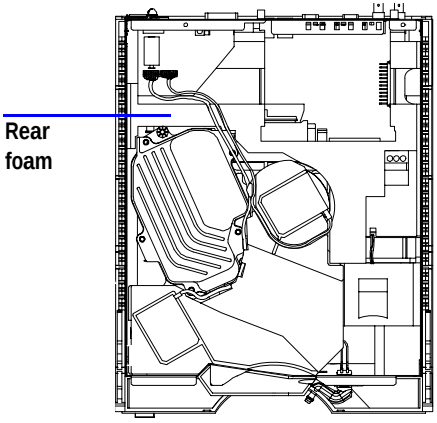
Preparations for this procedure:

- Turn OFF the detector.
- Disconnect the power cable.
- Remove the flow cell or disconnect the tubings from the flow cell.
- Remove detector from stack and place it on the workbench.
- Remove the covers as described in "Removing the Covers" on page 198.

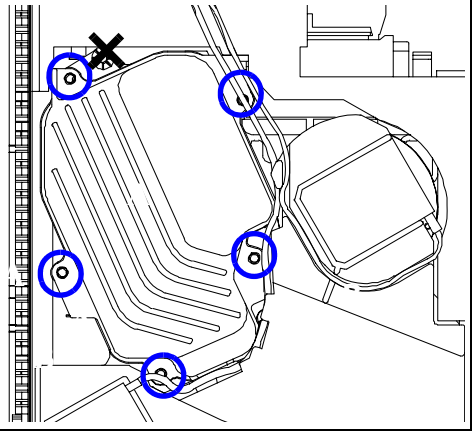
1 Disconnect the emission and the excitation cables and from the detector main board.



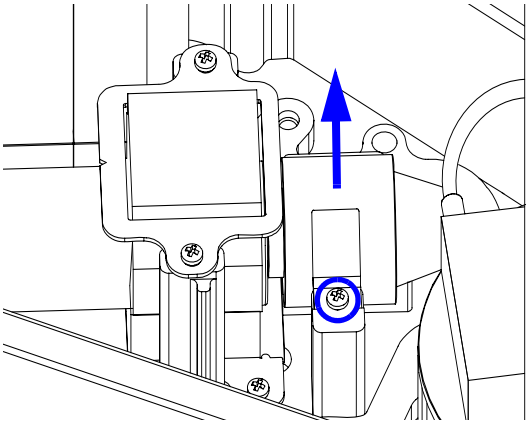
2 Carefully remove the rear foam.



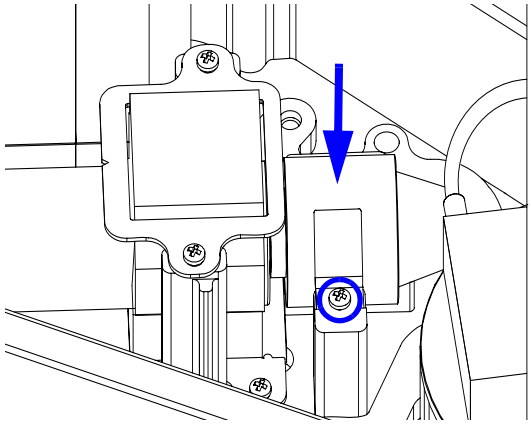
3 Using the 4 mm hexagonal key, unscrew the five screws, remove the cover and place it on the bench without turning it around. **DO NOT unscrew the grounding screw (marked with X)**.



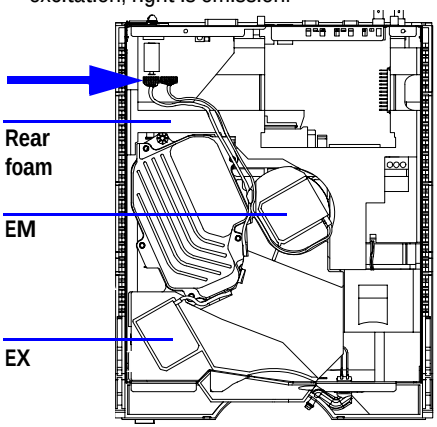
4 Using a 2.5 mm hexagonal key, loosen the screw and remove the condenser. There is a mechanical stop for the condenser.



5 Insert the new or cleaned condenser, shift it completely to its mechanical stop and fit the screw.



6 Replace the lamp housing cover and fix the screws. Carefully insert the rear foam. Reconnect the monochromator cables to the main board. Left is excitation, right is emission.



Next Steps:

- Replace the covers as described in “Replacing the Foam and the Top Cover” on page 149.
- Re-install the detector in the stack.
- Enter the replacement of the lamp in the maintenance logbook (this will reset the lamp counter).
- Turn the lamp ON.
- Perform a wavelength verification to check the correct positioning of the replaced assemblies, as described in chapter “Diagram of Light Path” on page 138.

Replacing the Lamp Cover Window

When required	If window is blocking the light throughput due to contamination
Tools required	Screwdriver POZI 1 PT3 Hexagonal screwdriver 2.5 mm, 100 mm long Hexagonal screwdriver 4 mm, 100 mm long
Parts required	Quartz Window 1000-0999

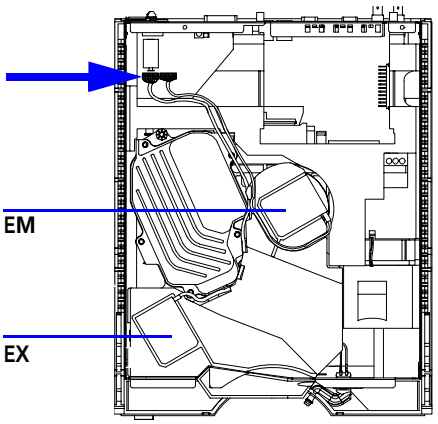
NOTE

When loosening or removing screws take care that they do not fall into the instrument. This may result in a complete removal of other assemblies.

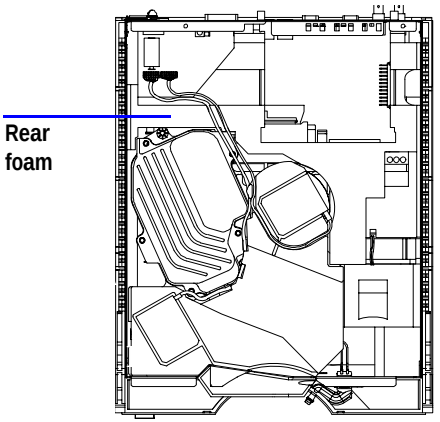
Preparations for this procedure:

- Turn OFF the detector.
- Disconnect the power cable.
- Remove the flow cell or disconnect the tubings from the flow cell.
- Remove detector from stack and place it on the workbench.
- Remove the covers as described in "Removing the Covers" on page 198.

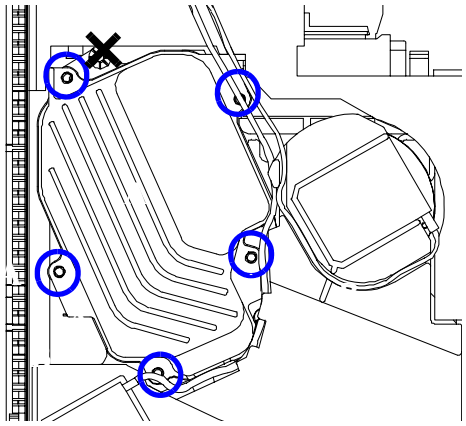
1 Disconnect the emission and the excitation cables and from the detector main board.



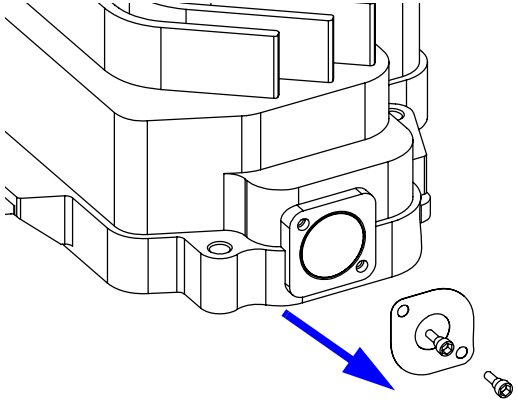
2 Carefully remove the rear foam.



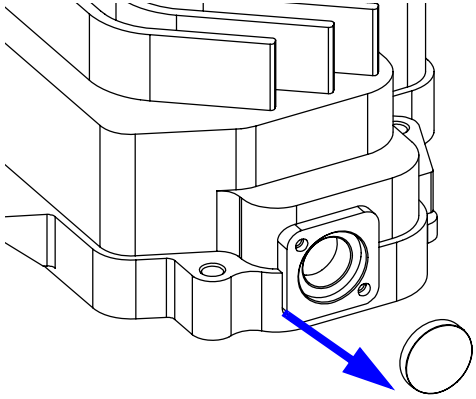
3 Using the 4 mm hexagonal key, unscrew the five screws, remove the cover and place it on the bench without turning it around. **DO NOT unscrew the grounding screw (marked with X)**.



4 Using the 2.5 mm hexagonal key, unscrew one screws and loosen the other one. Slide the window holder out of the window area.



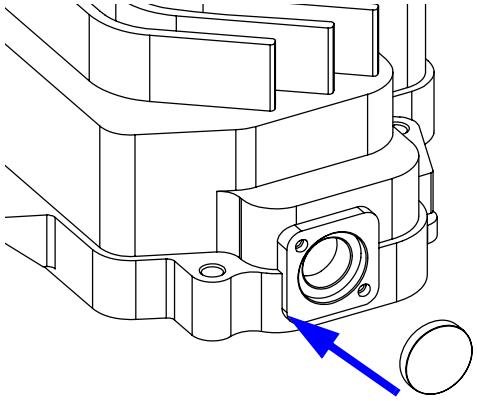
5 Use a toothpick to remove the window.



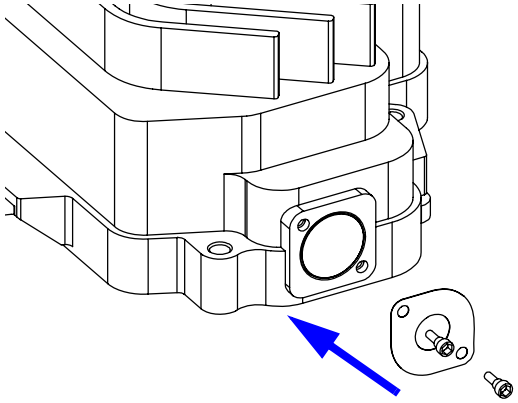
Note:

The quartz window may be cleaned with alcohol (e.g. ethanol).

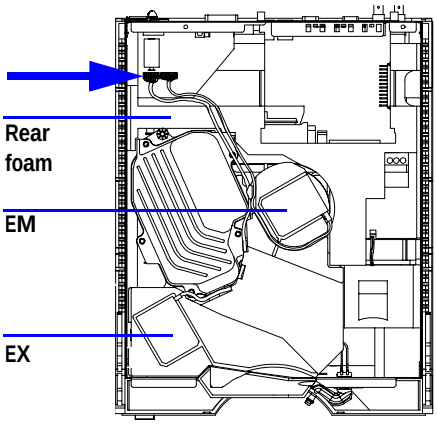
6 Insert the cleaned or new window.



7 Slide the window holder across the window and fix the screws.



8 Replace the lamp housing cover and fix the screws. Carefully insert the rear foam. Reconnect the monochromator cables to the main board. Left is excitation, right is emission.



Next Steps:

- Replace the covers as described in “Replacing the Foam and the Top Cover” on page149.
- Re-install the detector in the stack.
- Turn the lamp ON.

Exchanging the Main Board

When required	If detector main board is defective or for repair on other assemblies
Tools required	Screwdriver POZI 1 PT3 Flat screw driver Hexagonal wrenches 5 mm, 7 mm and 15 mm
Parts required	Detector main board (FLM) G1321-69500 (exchange assembly)

1 Turn OFF the lamp.

2 Switch OFF the module, and disconnect the cables.

3 Remove module from stack and place it on the workbench.

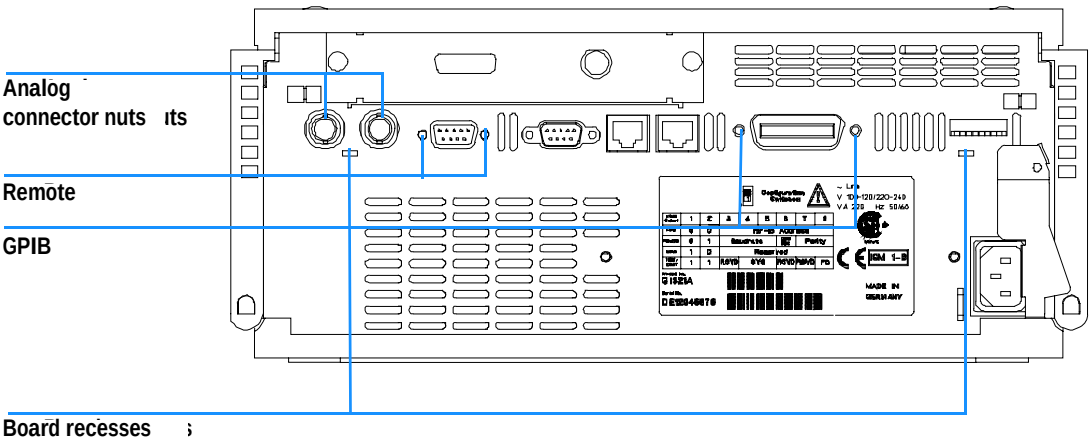


Figure 77 Unscrew Connectors from Board

4 Remove the front cover, top cover and top foam section, see "Removing the Covers" on page 198.

5 Use a 5-mm and 7-mm wrench to unscrew the REMOTE and the GPIB connector and a 15-mm wrench to unscrew the nuts of the ANALOG connectors.

6 Disconnect all connectors from the processor board.

NOTE When removing connectors, counter-hold with one hand on connector J12.

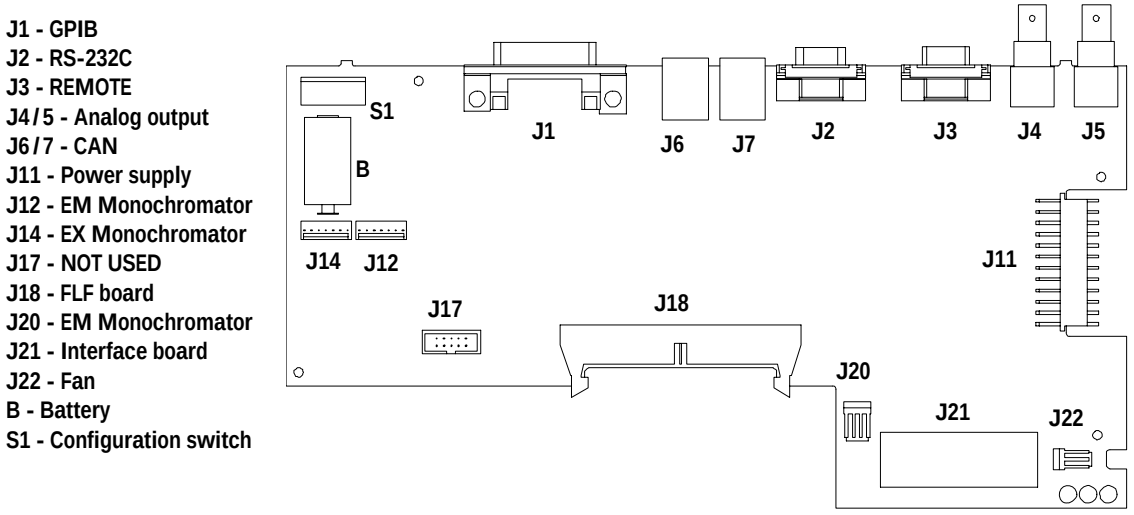


Figure 78 Location of Connectors on FLM Board

7 Remove the processor board. Place the board on the ESD kit.

8 On the new board check the switch setting of address switch S1, see "Setting the 8-bit Configuration Switch" on page 320.

NOTE An incorrect switch setting (for example, TEST / BOOT) may cause the module to revert to a basic mode (yellow or red flashing status light). In this case turn OFF the module, reset the address switches, and turn ON the module again.

9 Install the new processor board and reconnect the connectors. Assure that the board is fitted correctly into the board recess in the rear panel.

10 Refit the screws at the REMOTE and GPIB connectors and the nuts of the ANALOG connectors.

11 Reinstall the top foam section, top cover and front cover, see "Replacing the Foams and Covers" on page 254.

12 Replace detector into the stack and reconnect the cables.

NOTE If a new FLM board is installed, update the serial number information of the detector in the user interface, see procedure below.

13 Check the firmware revision of the module. If it is older than the current firmware revision, update the firmware using the standard firmware update procedure, see "Replacing the Detector's Firmware" on page 185.

Changing the Type and Serial Number

When required	If detector main board has been replaced.
Tools required	User interface
Parts required	none
Preparations	• Turn the detector on. • Start the user interface.

When the main board has to be replaced, the new board does not have serial number. For some modules (e.g. pumps or auto samplers) the type has to be changed (multiple usage boards). Use the information from the serial number plate of your module.

The changes become active after the reboot of the module.

Using the Agilent ChemStation

Module serial numbers are entered by typing specific commands on the command line at the bottom of the main user interface screen.

1 To enter a module serial number, type the following command into the command line:

```
print sendmodule$(lfld, "ser 'YYYYYYYYYY'")
```

Where: YYYYYYYYYY is the 10-character serial number of the module in question.

NOTE The first two characters are letters, which should be capitalized.

The reply line will respond with **RA 0000 SER** followed by the module serial number you just entered.

To change the type of the module use the following command:

```
print sendmodule$(lfld, "TYPE XXXXX")
```

Where: XXXXX is the 5-character product number of the module (e.g. G1314A).

WARNING **If you enter the wrong type, your module will not be accessible anymore. In such a case see "Using the Instant Pilot G4208A" on page 216 or "Using the Control Module G1323B" on page 217 for recovering.**

2 Turn OFF the detector, then ON again. Then, restart the Agilent ChemStation. If the serial number you have just entered is different than the original module serial number, you will be given the opportunity to edit the configure **1200 access** screen during the restart of the Agilent ChemStation.

3 After restart, the serial number/type you have just entered can be seen under the **Instrument** menu of the main user interface screen.

Using the Instant Pilot G4208A

1 Connect the Instant Pilot to the detector. Turn ON the detector.

2 On the Instant Pilot's Welcome screen, press **More**, then select **Maintenance**. Using the **up/down arrows**, select the detector where you have to change the product number or serial number.

3 Press **PN/SN**. This will display a screen where you can enter the product number and/or serial number.

4 Make your changes, using the information from the product label of your detector.

WARNING **If you enter the wrong type, your module might not be accessible anymore with the Agilent ChemStation and the Instant Pilot (unsupported module). In such a case follow the "Recover Instructions" on page 217.**

5 Press **OK** to highlight the complete command.

6 Press **Done** to transfer the information into the main board's memory. Press **Cancel** quit the process.

7 Turn the detector OFF then ON again. The Maintenance screen should display the correct serial number for this module.

8 If an Agilent ChemStation is also connected, restart the Agilent ChemStation now as well.

Recover Instructions

1 Turn off the detector.

2 Change the 8-bit Configuration Switch to Resident (see "Stay-Resident Settings" on page 324).

3 Turn the detector on.

4 Re-do steps 2 to 5 of "Using the Instant Pilot G4208A" on page 216 and correct the type information. Enter the product number without "-R"

5 Turn the detector off.

6 Change the 8-bit Configuration Switch back to default settings (see "Setting the 8-bit Configuration Switch" on page 320).

7 Turn the detector ON again. The Maintenance screen should display the correct type for this module.

Using the Control Module G1323B

1 Connect the control module to the detector. Turn ON the detector.

2 On the control module, press **System** (**F5**), then **Records** (**F4**). Using the **up/down arrows**, make sure that the detector is highlighted.

3 Press **FW Update** (**F5**), then **m.** This will display a box which says **Update Enter Serial#**.

4 Press **Enter**. This will display the box labeled **Serial#**.

5 Letters and numbers are created using the up and down arrows. Into the box labeled Serial#, enter the 10-character serial number for the detector. When the 10-character serial number is entered, press **Enter** to highlight the complete serial number. Then, press **Done** (**F6**).

6 Turn the detector OFF then ON again. The Records screen should display the correct serial number for this module.

7 If a Agilent ChemStation is also connected, restart the Agilent ChemStation now as well.

To change the product number go to the **System** screen.

1 Press **Tests (F3)** and select the detector and press **Enter**.

2 While in the Tests screen, press **m.m** (m dot m).

3 From the box now displayed, select the **Command**, and press **Enter**.

4 Into the box labeled **Nester** (instruction), enter the command **TYPE XXXXX**.

Letters and numbers are created using the up and down arrows. XXXXX is the 5-character product number of the module being changed. There must be a space between the word TYPE and the product number.

Examples: **TYPE G1321A** to configure as a FLD.

WARNING **If you enter the wrong type, your module might not be accessible anymore with the Agilent ChemStation. In such a case re-enter the TYPE command correctly.**

5 Now, press the **Execute** key. Below the box, a reply line should then say:

Reply RA 0000 TYPE "XXXXX" (XXXXX is what you just entered)

Turn the detector off, then on again. Turn on should be normal. In the **Records** screen, the product# column should indicate the module you just entered. If an Agilent ChemStation is also connected, re-boot it now.

Exchanging the Fan

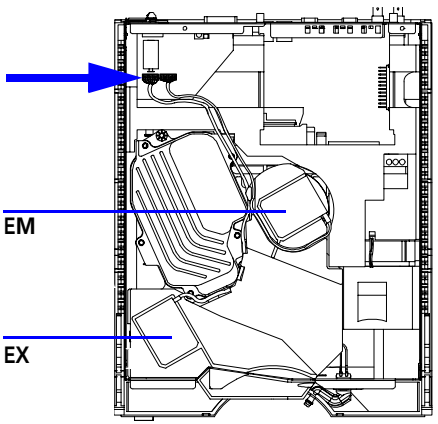
When required	If the fan is defective or noisy
Tools required	Screwdriver POZI 1 PT3
Parts required	Fan assembly 3160-1017

CAUTION The fan must be installed in the correct orientation to ensure optimum cooling and operation of the detector.

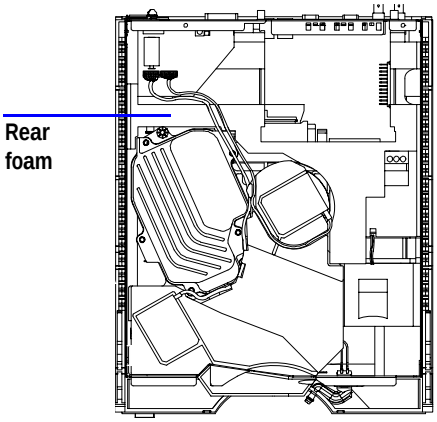
Preparations for this procedure:

- Turn OFF the detector.
- Disconnect the power cable.
- Remove the flow cell.
- Remove detector from stack and place it on the workbench.
- Remove the covers as described in "Removing the Covers" on page 198.

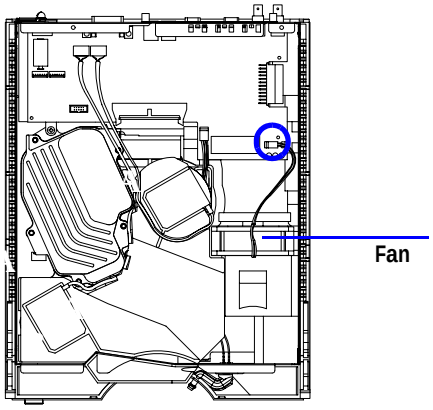
1 Disconnect the emission and the excitation cables and from the detector main board.



2 Carefully remove the rear foam.



3 Lift the main board slightly. Bring the fan cable up and disconnect the fan cable. Pull out the fan.

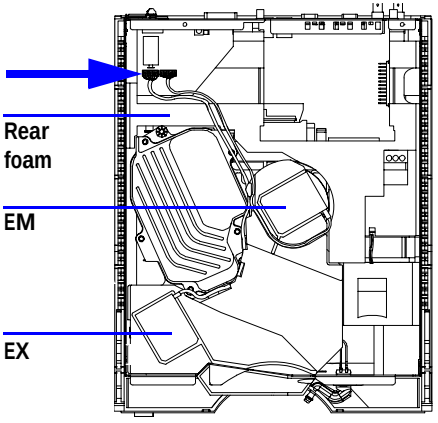


4 When the fan is inserted assure that the air flow is from the rear towards the front of the detector.

Insert the fan.

Lift the main board slightly. Reconnect the fan cable and route it underneath the board.

5 Carefully insert the rear foam. Reconnect the monochromator cables to the main board. Left is excitation, right is emission.



Next Steps:

- Replace the covers as described in "Replacing the Foams and Covers" on page 254.
- Re-install the detector in the stack.

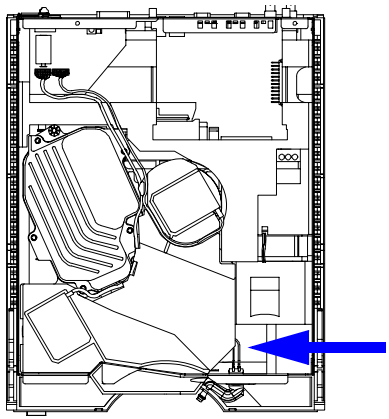
Exchanging the Leak Sensor or Leak Panel

When required	If defective
Tools required	Screwdriver POZI 1 PT3 Flat screwdriver
Parts required	Leak sensor assembly 5061-3356 Leak Pan G1321-40511

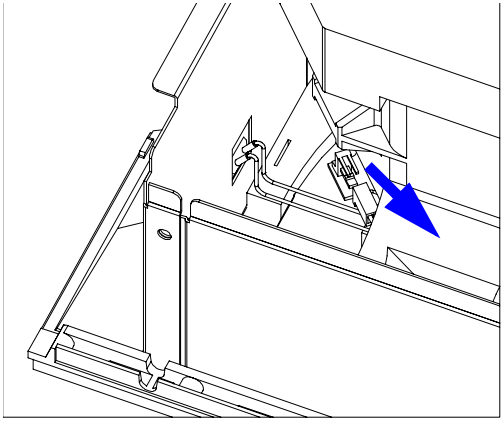
Preparations for this procedure:

- Turn OFF the detector.
- Disconnect the power cable.
- Remove the flow cell.
- Remove detector from stack and place it on the workbench.
- Remove the covers as described in “Removing the Covers” on page 198.
- Remove the flow cell.

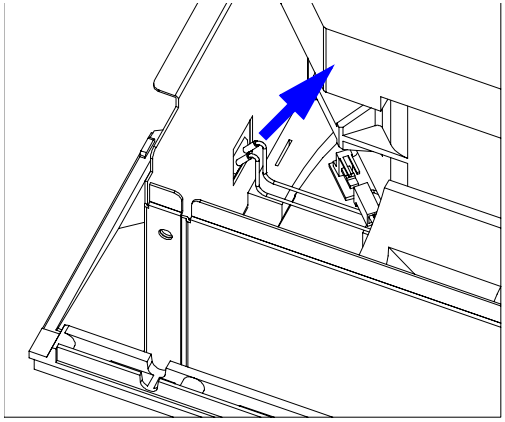
1 Locate the leak sensor cable.



2 Disconnect the leak sensor cable using your left hand.



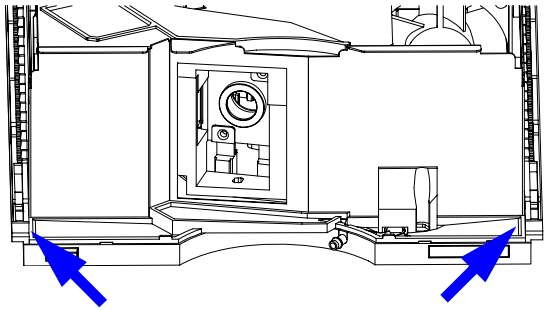
3 Pull out the leak sensor.



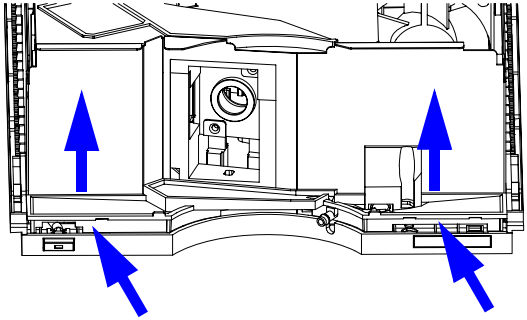
Note:

If you want to replace just the leak sensor, continue with step 8.

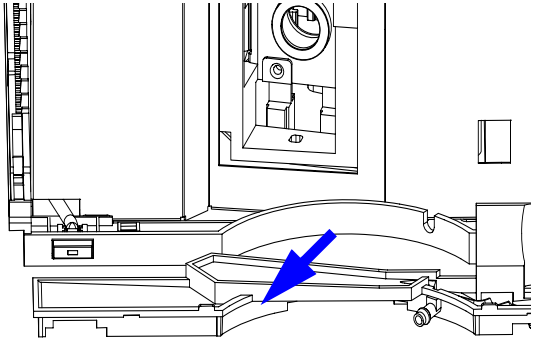
4 Using flat screw driver, release the leak base (two clasps).



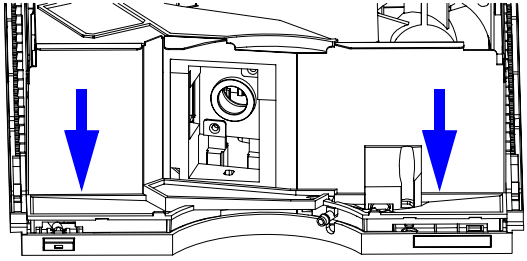
5 Use a flat screw driver to lift the leak base from the base.



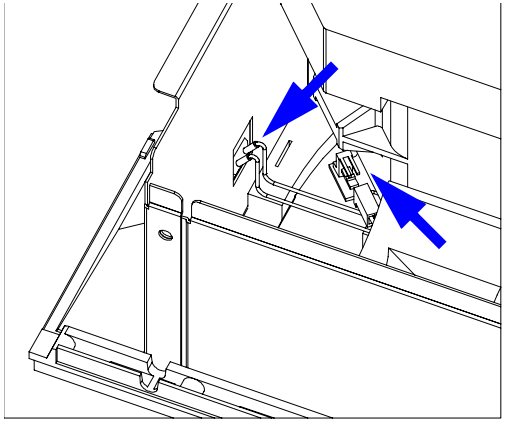
6 Remove the leak base completely.



7 Replace the leak pan into the cabinet bottom. Press the leak pan completely down into its recess.



8 Replace the leak sensor using your left hand and reconnect the connector.



Next Steps:

- Replace the covers as described in "Replacing the Foams and Covers" on page 254.
- Re-install the flow cell and the front panel
- Re-install the detector in the stack.

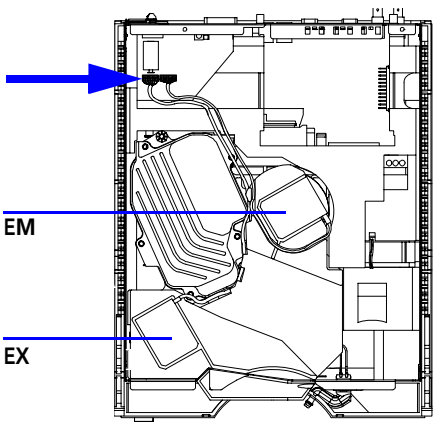
Replacing the EM-Condenser or Cutoff-Filter

When required	If light throughput is too low
Tools required	Screwdriver POZI 1 PT3 Hexagonal key 2.5 mm
Parts required	Condenser assembly EM 1000-1124 Cutoff filter 295 nm 5062-8530

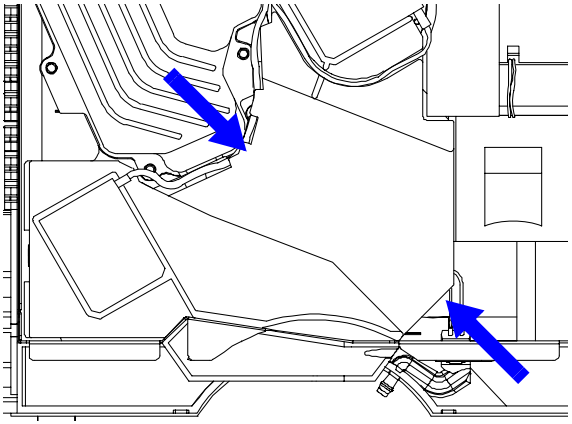
Preparations for this procedure:

- Turn OFF the detector.
- Disconnect the power cable.
- Remove the flow cell.
- Remove detector from stack and place it on the workbench.
- Remove the covers as described in "Removing the Covers" on page 198.
- Remove the flow cell.

1 Disconnect the emission and the excitation cables and from the detector main board.

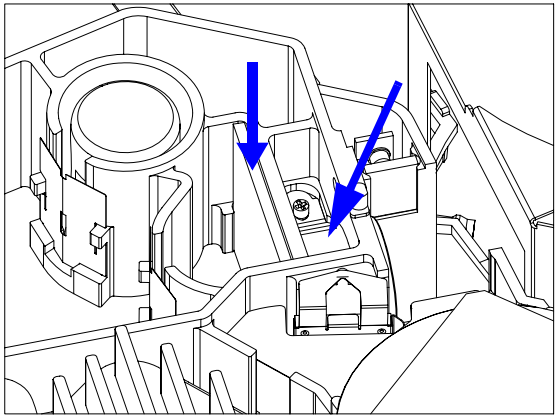


2 Carefully remove the front foam holding the foam as shown.



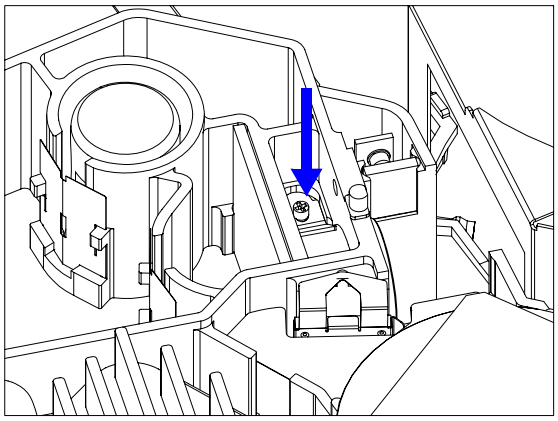
Note:

Locate the condenser assembly and the cutoff filter.

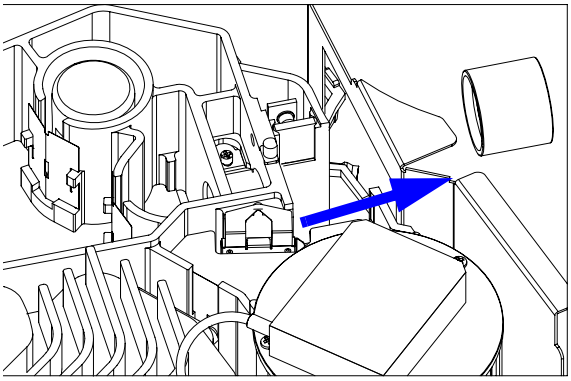


3 The cutoff filter can be replaced just by sliding it out and back in.

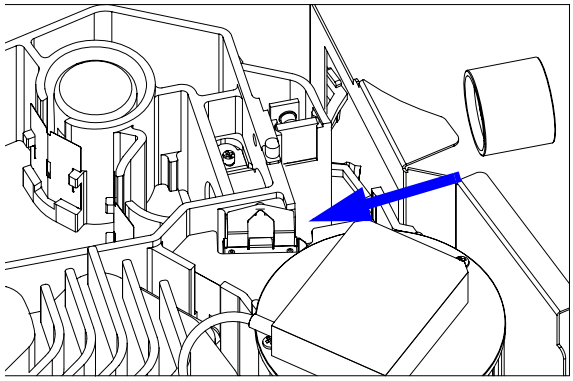
4 Use a 2.5 mm hexagonal key to loosen the screw.



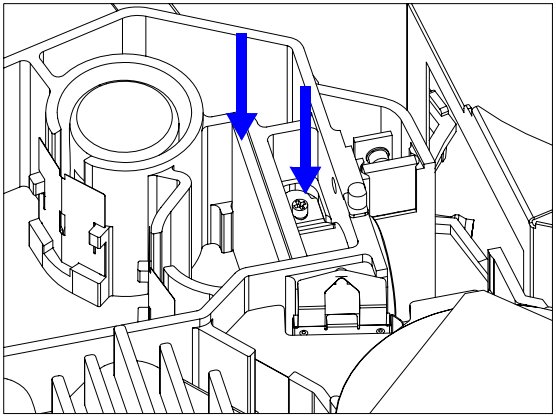
5 Slide out the condenser assembly and place it in a safe location.



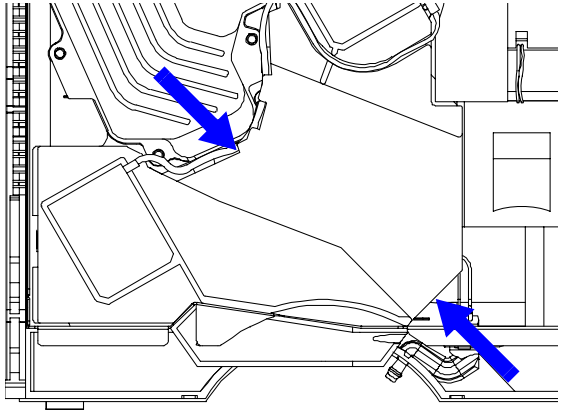
6 Slide in the condenser assembly completely with the larger lens diameter towards the cutoff filter.



7 Use a 2.5 mm hexagonal key to fix the screw. Replace the cutoff filter.



8 Carefully replace the front foam.



Next Steps:

- Replace the covers as described in "Replacing the Foams and Covers" on page 254.
- Re-install the flow cell and the front panel.
- Re-install the detector in the stack.
- Turn the lamp ON.
- Perform a wavelength verification to check the correct positioning of the replaced assemblies, as described in chapter "Diagram of Light Path" on page 138.

Replacing the Mirror Assembly

When required	If reflection is too low (contaminated)
Tools required	Screwdriver POZI 1 PT3 A pair of tweezers
Parts required	Mirror assembly 1000-1000

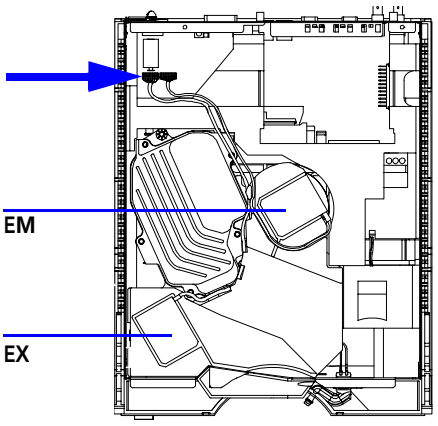
NOTE DO NOT touch the surface of any optical component during this procedure. This will reduce the reflection efficiency.

NOTE One side of the mirror is marked “R” representing the rear side.

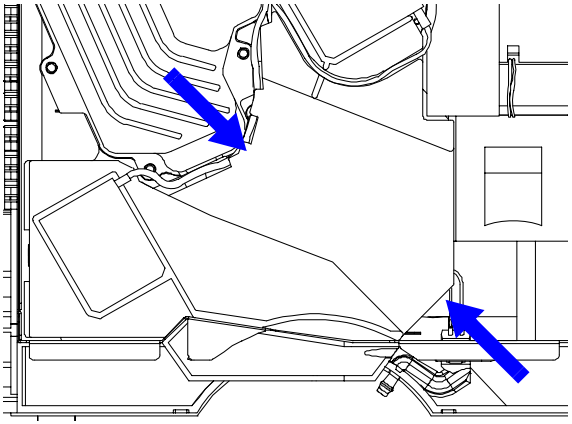
Preparations for this procedure:

- Turn OFF the detector.
- Disconnect the power cable.
- Remove the flow cell.
- Remove detector from stack and place it on the workbench.
- Remove the covers as described in “Removing the Covers” on page 198.

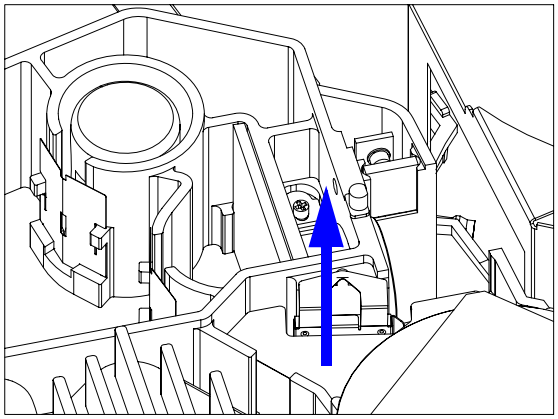
1 Disconnect the emission and the excitation cables and from the detector main board.



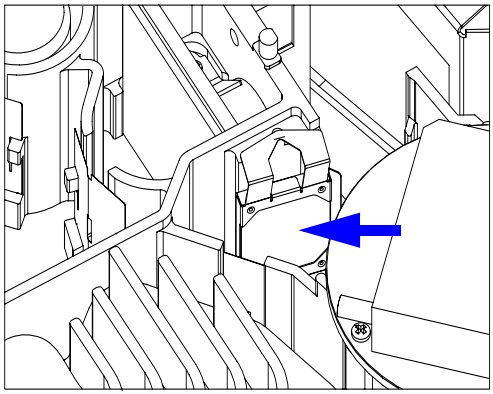
2 Carefully remove the front foam holding the foam as shown.



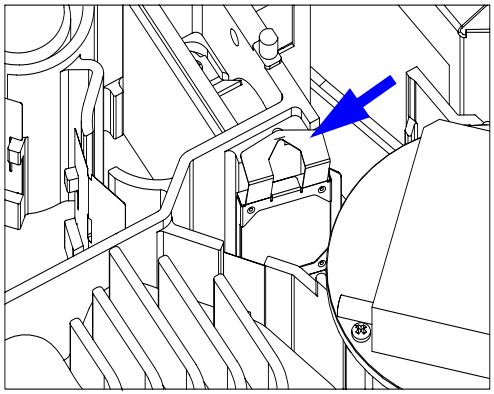
3 Remove the UV protector.



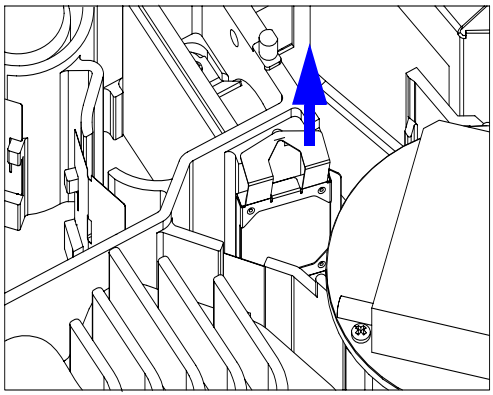
4 Locate the mirror assembly.



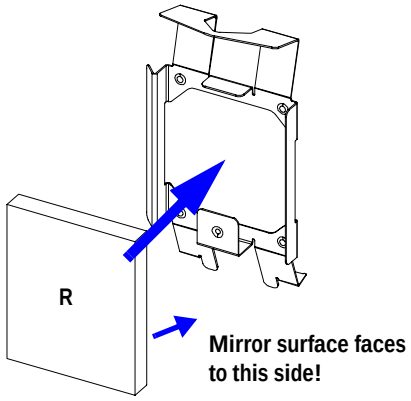
5 Use a pair of tweezers to lift off the top of the mirror holder.



6 Remove the mirror assembly.



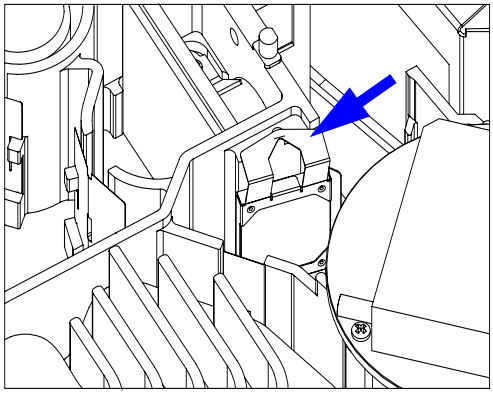
7 Insert the new mirror into the holder. The mirror surface faces towards the light. The back side is marked as shown.



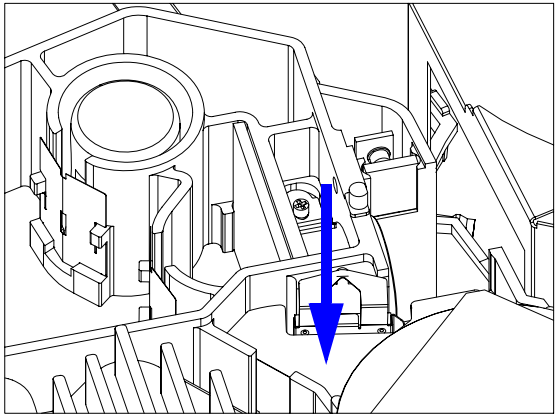
Note:

If the mirror is inserted in the opposite direction, the detector cannot be aligned correctly.

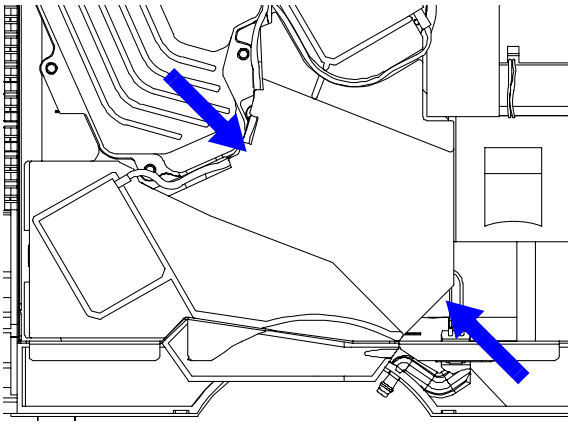
8 Replace the mirror assembly and lock the top over the pin.



9 Replace the UV protector.



10 Replace the front foam.



Next Steps:

- Replace the covers as described in “Replacing the Foams and Covers” on page 254.
- Re-install the flow cell and the front panel
- Re-install the detector in the stack.
- Turn the lamp ON.
- Perform a wavelength verification to check the correct positioning of the replaced assemblies, as described in chapter “Diagram of Light Path” on page 138.

Replacing the Slits

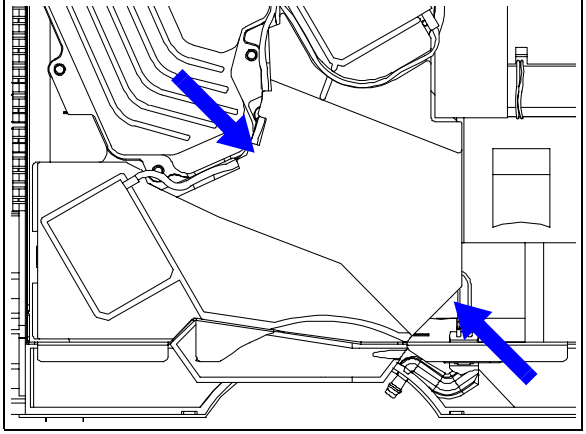
When required	If required.
Tools required	Screwdriver POZI 1 PT3 A pair of tweezers
Parts required	Slit EX G1321-08103 Slit EM G1321-08102 (also used for PMT)

NOTE DO NOT touch the surface of any optical component during this procedure. This will deteriorate the reflection process.

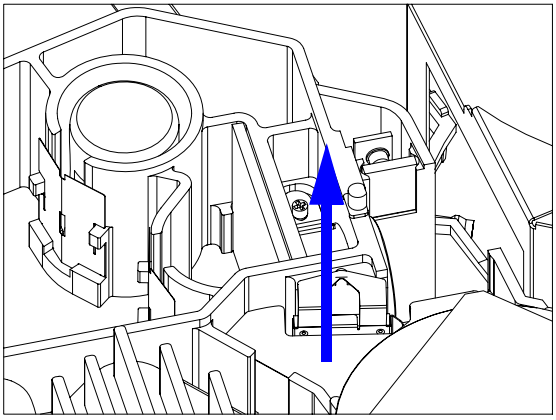
Preparations for this procedure:

- Turn OFF the detector.
- Disconnect the power cable.
- Remove the flow cell.
- Remove detector from stack and place it on the workbench.
- Remove the covers as described in "Removing the Covers" on page 198.

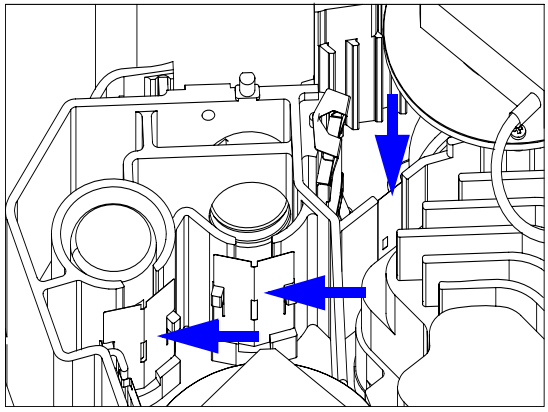
1 Carefully remove the front foam holding the foam as shown.



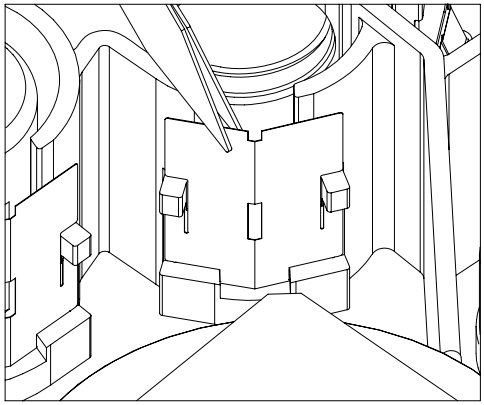
2 Remove the UV protector.



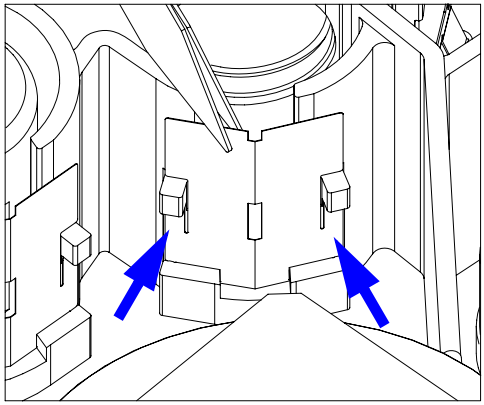
3 Locate the three slits (from left to right: PMT, EM, EX).



4 Use a pair of tweezers to replace the slit.



5 Put slight pressure on to the spring part of the slit.



Next Steps:

- Replace the UV protector and the front foam.
- Replace the covers as described in "Replacing the Foams and Covers" on page 254.
- Re-install the flow cell and the front panel
- Re-install the detector in the stack.
- Turn the lamp ON.
- Perform a wavelength verification to check the correct positioning of the replaced assemblies, as described in chapter "Diagram of Light Path" on page 138.

Removing the Optical Unit

When required	For following repairs: power supply, FLL board, Trigger Pack/FLF board and PMT.
Tools required	Screwdriver POZI 1 PT3 Hexagonal key, 4 mm
Parts required	Optical unit G1315-69002 (exchange assembly, comes with foam) or individual parts depending on the following procedures

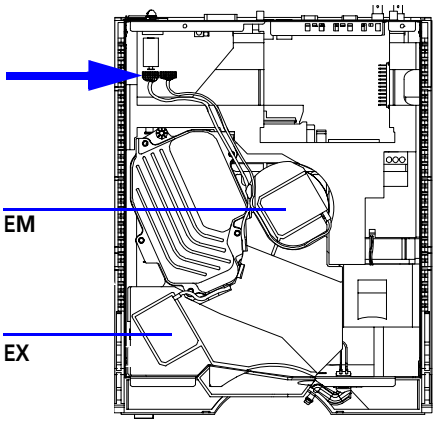
CAUTION When working on the optical unit, a clean workbench with ESD protection mat must be available. Otherwise optical components or electronic boards may be damaged.

NOTE DO NOT remove the foam from the optical unit unless it is required during a procedure. Otherwise parts can fall out.

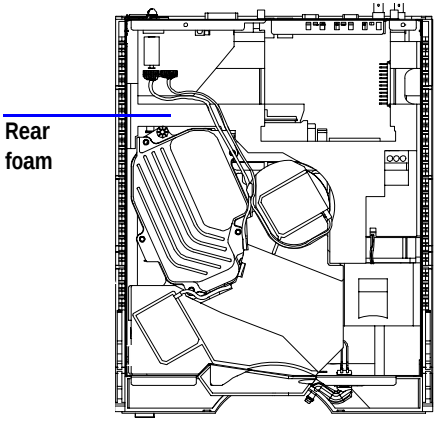
Preparations for this procedure:

- Turn OFF the detector.
- Disconnect the power cable.
- Disconnect capillaries.
- Remove detector from stack and place it on the workbench.
- Remove the covers as described in "Removing the Covers" on page 198.
- Remove the flow cell.

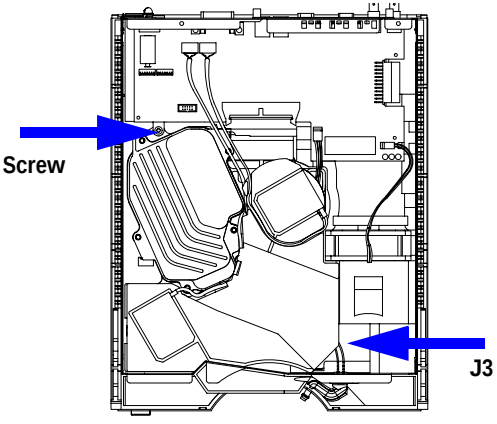
1 Disconnect cables (EM monochromator) and (EX monochromator) from the detector main board (FLM).



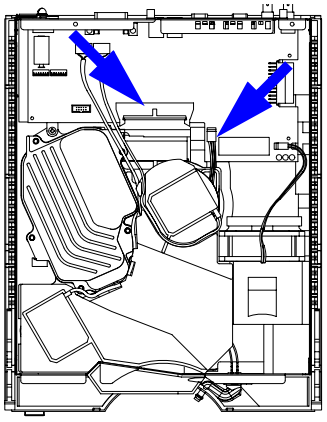
2 Carefully remove the rear foam (A). Leave the other foam on the assembly.



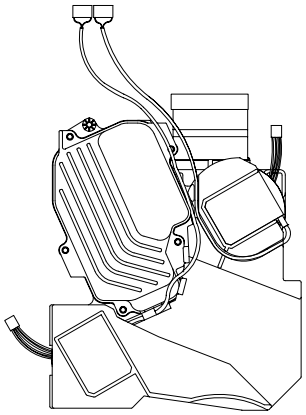
3 Disconnect the leak sensor cable (J3) and unscrew the grounding screw.



4 Disconnect the optical unit cable (J18) and the EM motor cable (J20).



5 Remove the optical unit and place it on the bench (ESD protection required).



Next Steps:

- Depending on the replacement you want to perform, proceed to the following procedures.

Replacing PMT and / or FLF board

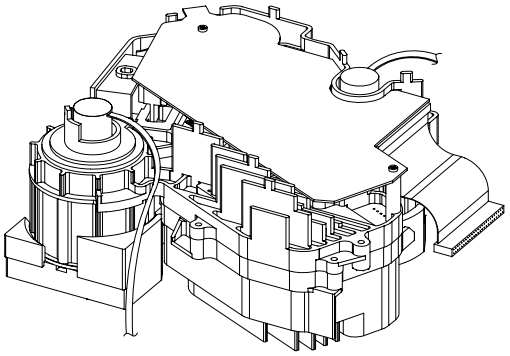
When required	If defective or if application specific PMT is required.
Tools required	Screwdriver POZI 1 PT3 Hexagonal key, 4 mm and 2.5 mm Tweezers
Parts required	Photo-multiplier Tube (PMT) 1970-0201 Photo-multiplier Tube (PMT) for other application ranges, please contact Hamamatsu dealers. Alternative PMTs are either R928HA (185 to 900 nm) or R3788HA (185 to 750 nm). No other PMTs are recommended. FLF Board G1321-69531 (requires firmware revision A.04.06 or above), FLR Reference Diode board G1321-66533

CAUTION When working on the optical unit, a clean workbench with ESD protection mat must be available. Otherwise optical components or electronic boards may be damaged.

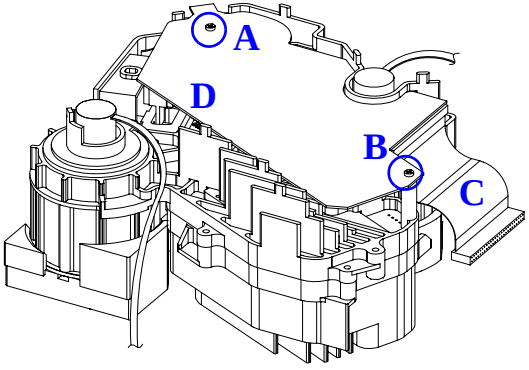
Preparations for this procedure:

- Optical unit has been removed as described in "Removing the Optical Unit" on page 233.
- The foam must be in place. Otherwise cutoff filter and diffuser will fall out.

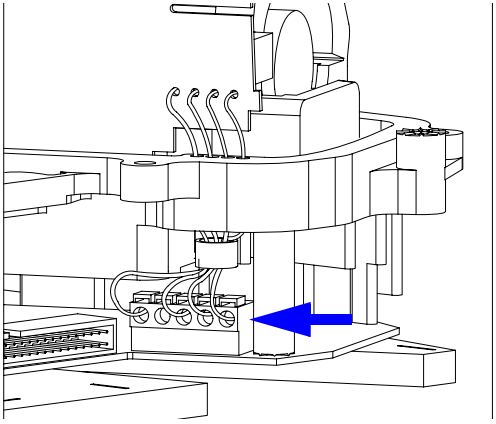
1 Place the optical unit upside down on the ESD protection mat.



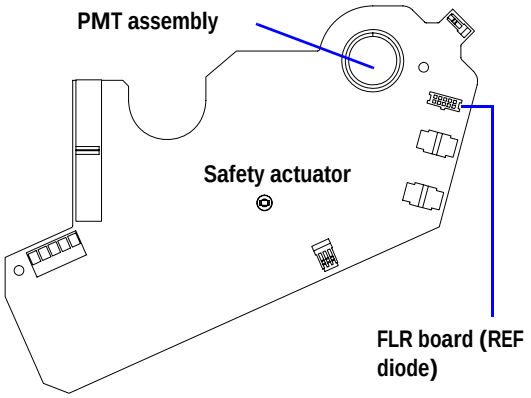
2 Unscrew screws A and B and disconnect connectors C and D.



3 Disconnect the wires from the connector. Use a flat screw driver to release the wires.



4 Remove the FLF board carefully in an upwards motion and place it on an ESD protected mat.

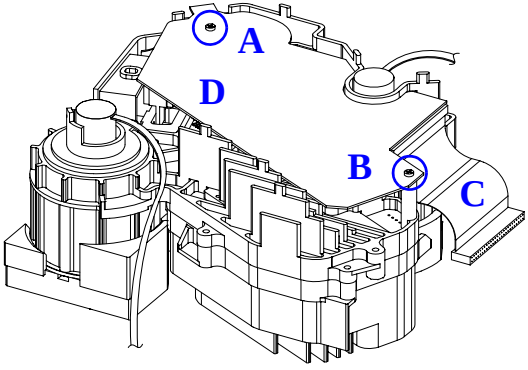


5 If you need to replace either the board or the PMT assembly, remove the PMT assembly from its socket.

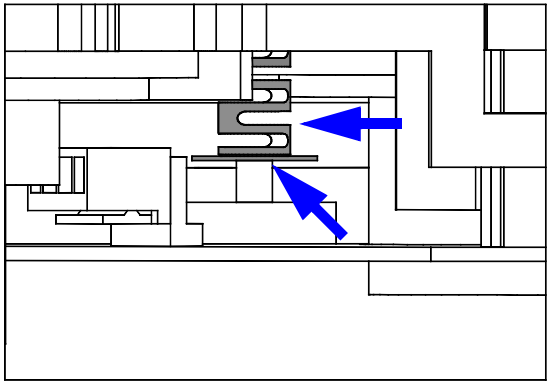
6 Replace the PMT assembly or the FLF board.

7 Re-install the PMT assembly in the socket.

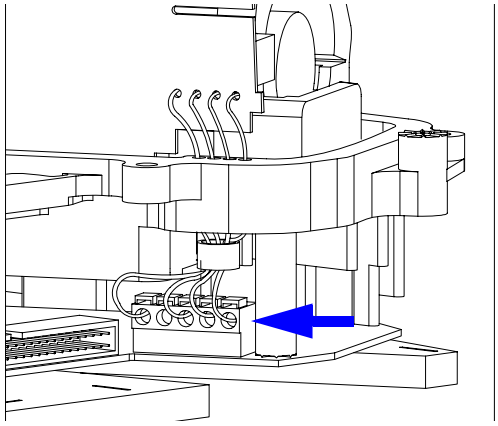
8 Replace the FLF board and fix the screws (A and B) and connectors (C and D).



9 Check that the safety switch is positioned correctly. Look through board and casting.



10 Reconnect the wires to the connector. Use a flat screw driver to release the wire socket.



11 Put the wires into the released contacts using the tweezers.

Order of wires (left to right):

green
empty
brown
yellow
white

Next Steps:

- Reinstall the optical unit as described in "Installing the Optical Unit" on page 252.
- If you replaced a G1321-66501 FLF board by a newer FLF board, update the FLD firmware to revision to latest version (to make it compatible).

Replacing FLL board and Trigger Pack

When required	If defective
Tools required	Screwdriver POZI 1 PT3 Hexagonal key, 4 mm and 2.5 mm
Parts required	FLL Board G1321-66512 (**includes** trigger pack)

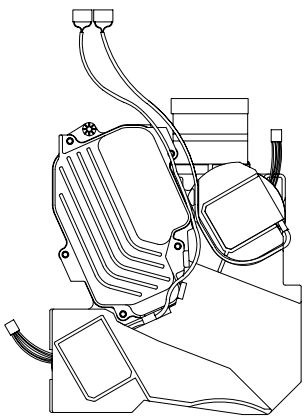
CAUTION When working on the optical unit, a clean workbench with ESD protection mat must be available. Otherwise optical components or electronic boards may be damaged.

WARNING **The instrument should not be operated without Xenon flash lamp. Otherwise the Triggerpack electronics will be damaged.**

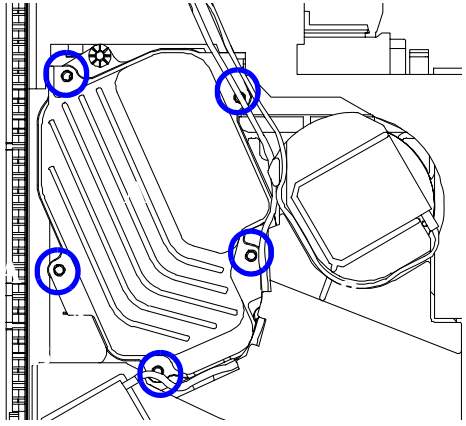
Preparations for this procedure:

- Optical unit has been removed as described in "Removing the Optical Unit" on page 233.
- The foam must be in place. Otherwise cutoff filter and diffuser will fall out.

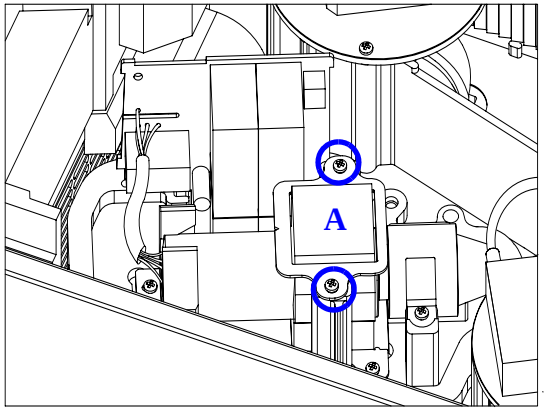
1 Place the optical unit on the ESD protection mat.



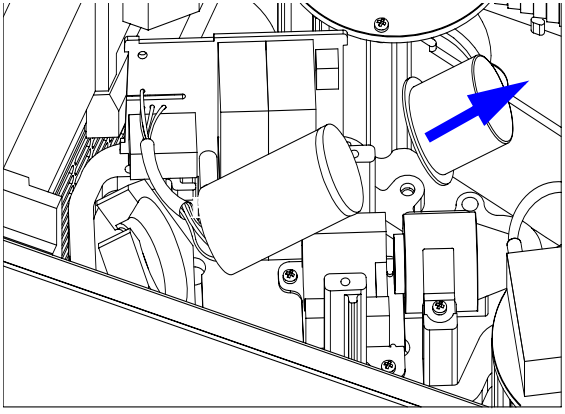
2 Using the 4 mm hexagonal key, unscrew the five screws and lift the cover.



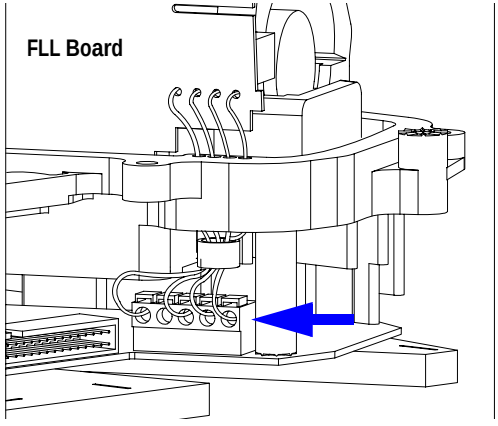
3 Using the 2.5 mm hexagonal key, unscrew the two screws and remove the pad (A).



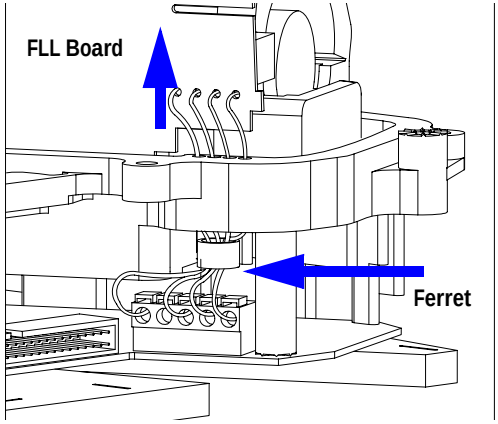
4 Remove the lamp as described in "Exchanging the Lamp" on page 201.



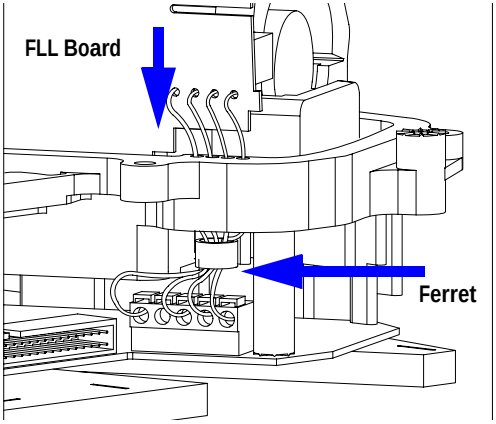
5 Disconnect the wires from the connector. Use a flat screw driver to release the wires.



6 Carefully pull off the ferret from the wires and keep it in a safe place. Then pull out the FLL board.



7 Insert the new FLL board. Fit the wires through the holes and the Ferret.

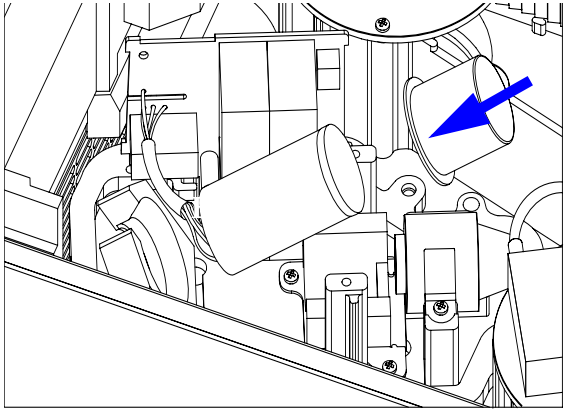


8 Put the wires into the released contacts using the tweezers.

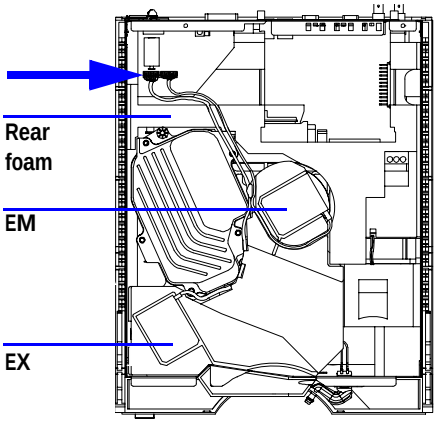
Order of wires (left to right):

green
empty
brown
yellow
white

9 Reinstall the lamp as described in "Exchanging the Lamp" on page 201.



10 Replace the lamp housing cover. Insert the optical unit into its location and carefully insert the rear foam (A). Then reconnect the cables.



Next Steps:

- Replace the covers as described in "Replacing the Foams and Covers" on page 254.
- Re-install the detector in the stack.
- Reset the lamp counter as described in the user interface documentation.
- Turn the lamp ON.
- Perform a wavelength verification to check the correct positioning of the replaced assemblies, as described in chapter "Diagram of Light Path" on page 138.

Replacing the EM and EX Grating

When required	If defective
Tools required	Screwdriver POZI 1 PT3 Hexagonal key, 4 mm and 2.5 mm
Parts required	EM Grating assembly G1321-60004 (**includes** protection ring) EX Grating assembly G1321-60003 (**includes** protection ring)

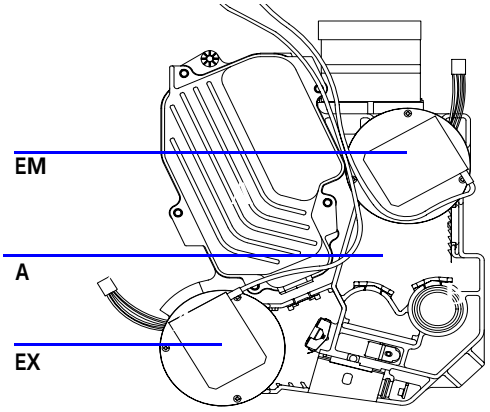
CAUTION When working on the optical unit, a clean workbench with ESD protection mat must be available. Otherwise optical components or electronic boards may be damaged.

NOTE The grating assembly is shipped with a special transport packaging and includes a protection ring. DO NOT touch the grating surface and DO NOT bend the protection ring.

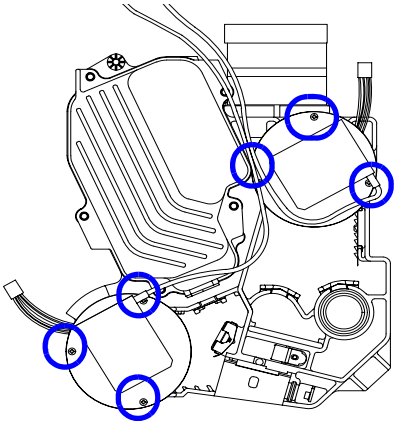
Preparations for this procedure:

- Optical unit has been removed as described in "Removing the Optical Unit" on page 233.
- The cables from the position encoders (at the rear of the EM or EX grating assembly) should be disconnected.
- Disconnect the encoder cable of the EX-monochromator from FLF board.

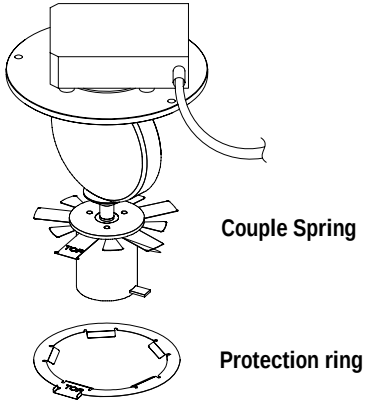
1 Place the optical on the ESD protection mat and remove the front foam (A). Locate the EM and EX grating assembly.



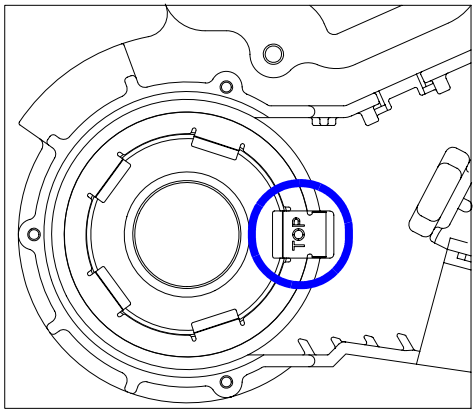
2 Use a 2.5 mm hex key to unscrew the 3 screws on the assembly you want to remove.



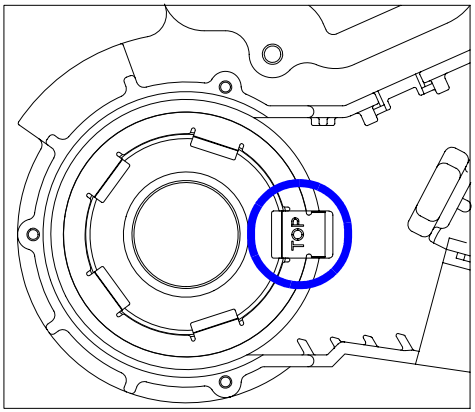
3 Carefully remove the grating assembly and the protection ring from its location.



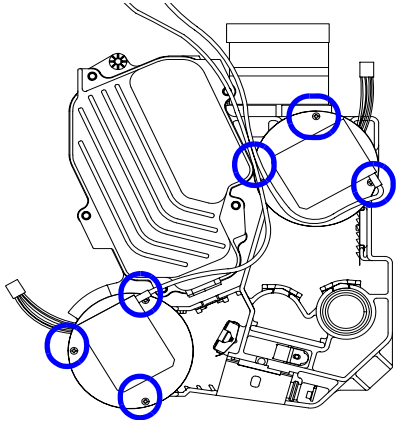
4 Insert the new protection ring into its position. Do not bend the protection ring.



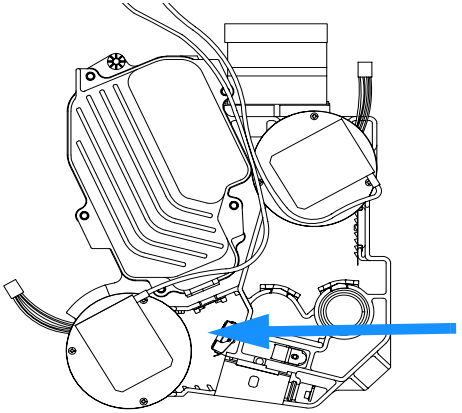
5 For reinstallation route the cable first through the hole. Carefully insert the grating into its position while pulling the cable slightly from below. The couple spring has to be clipped into the protection ring (TOP mark)!



6 Bring the top of the assembly into the position shown below and fix the 3 screws.



7 Assure the UV protector cover is installed. Replace the foam on the optical unit.



Next Steps:

- Reconnect the encoder cable of the EX-monochromator to FLF board.
- Reinstall the optical unit as described in “Installing the Optical Unit” on page 252.
- Enter the replacement in the maintenance logbook.
- Reset the monochromator settings to default using the diagnose functions.
- In this case “**Wavelength Calibration Lost**” is displayed and the instrument stays in **Not Ready** condition until you perform a wavelength calibration.
- Perform a wavelength verification to check the correct positioning of the replaced assemblies, as described in chapter “Diagram of Light Path” on page 138.

Exchanging the Power Supply

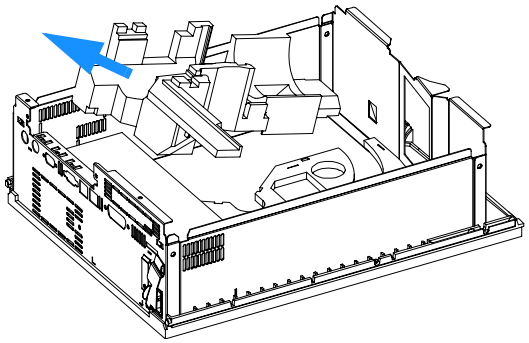
When required	If defective
Tools required	Screwdriver POZI 1 PT3 Wrench 1/4 inch Wrench 5 mm Wrench 7 mm
Parts required	Power supply 0950-2528

NOTE The repair of the power supply assembly comprises exchanging the complete assembly. No serviceable parts are inside.

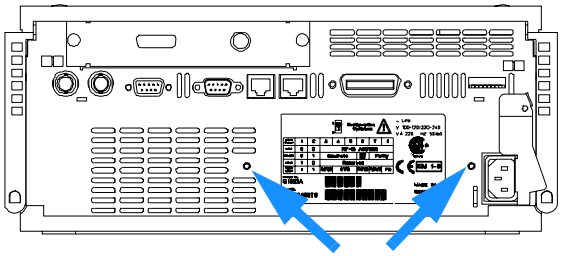
Preparations for this procedure:

- Turn OFF the lamp.
- Switch OFF the detector, and disconnect the cables.
- Remove the detector from the stack and place it on the workbench.
- Remove the front cover and top cover, see “Removing the Covers” on page 198.
- Remove the processor board, see “Exchanging the Main Board” on page 212.
- Remove the fan assembly, see “Exchanging the Fan” on page 219.
- Remove the optical unit, see “Removing the Optical Unit” on page 233.

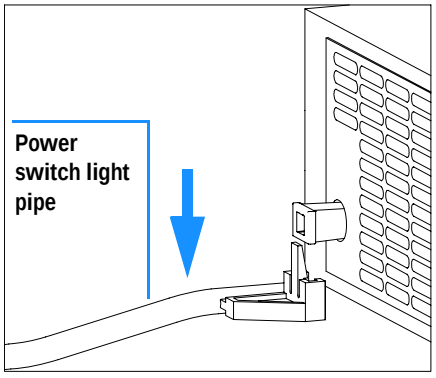
1 Carefully remove the bottom foam piece by sliding it out towards the rear.



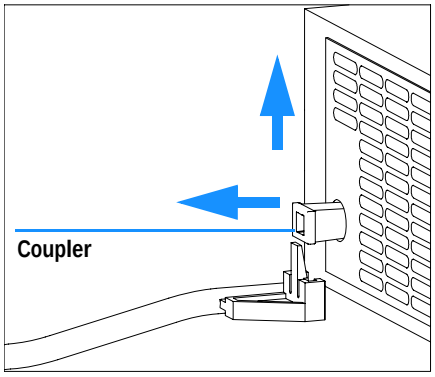
2 Unscrew the power supply at the rear of the module.



3 Press down the power switch light pipe to remove it from the coupler.

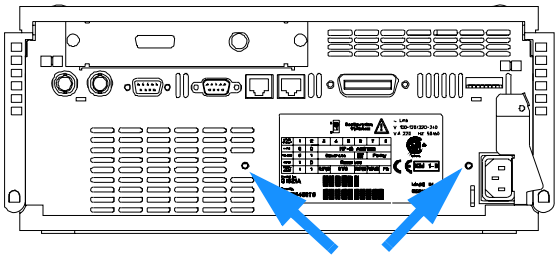


4 Remove the power supply completely.
Re-use the coupler on the new power supply.

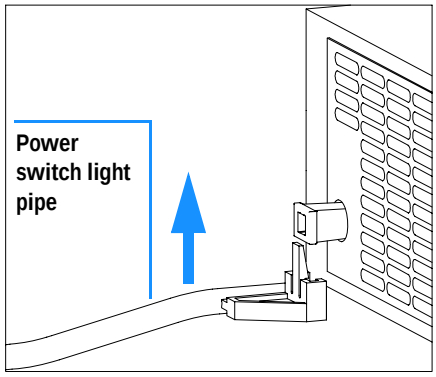


The repair of the power supply assembly is comprised of exchanging the complete assembly. No serviceable parts are inside.

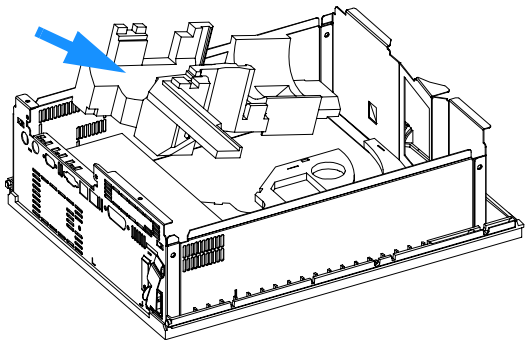
5 Insert the power supply into its location and fix it with the screws at the rear panel.



6 Press down and clip in the power switch light pipe into the power supply.



7 Reinstall bottom foam piece. Slide it in underneath the leak drain.



8 Reinstall the processor board, see “Exchanging the Main Board” on page 212.

9 Reinstall the fan assembly, see “Exchanging the Fan” on page 219.

10 Reinstall the optical unit, see “Installing the Optical Unit” on page 252.

11 Reinstall the front cover, top cover, see “Replacing the Foams and Covers” on page 254.

12 Replace detector into the stack.

13 Reconnect the power cable and turn on the detector.

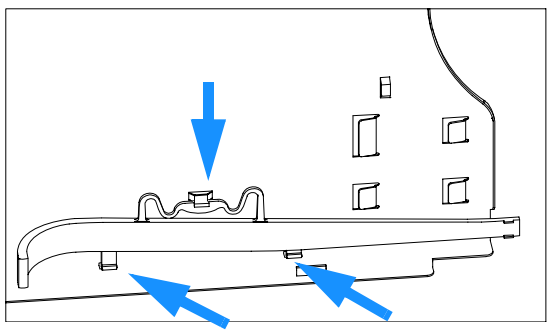
Replacing Status Light Pipe

When required	If part was broken or removed
Tools required	Screwdriver POZI 1 PT3
Parts required	Status light pipe 5041-8384

Preparations for this procedure:

- Remove the front cover and top cover, see "Removing the Covers" on page 198.

1 The status light pipe is clipped into the top cover.



2 Replace the top cover, see "Replacing the Foams and Covers" on page 254.

3 Replace the detector into the stack and reconnect the cables and capillaries.

4 Turn ON the detector.

Installing the Optical Unit

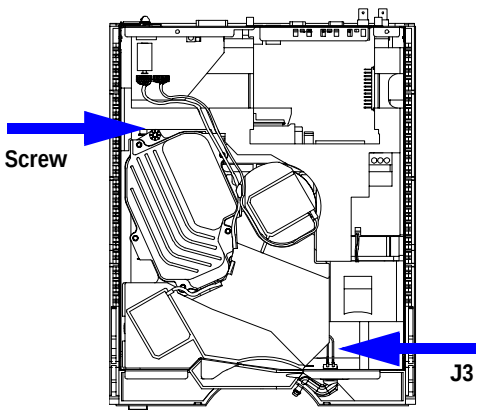
When required	When all repairs on the optical unit have been completed
Tools required	Screwdriver POZI 1 PT3 Hexagonal key, 4 mm

WARNING **For safe operation make sure that the ground cable is reconnected.**

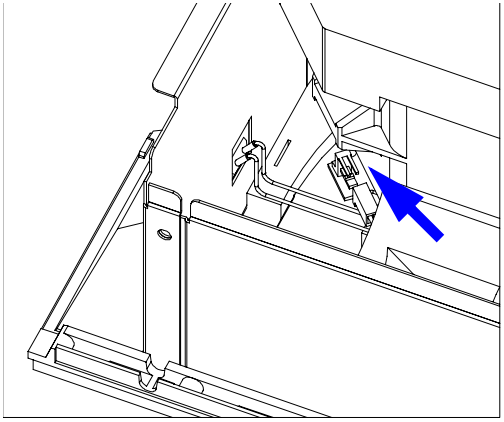
Preparations for this procedure:

- All previous work has been completed.
- Power supply has been installed.
- Fan has been installed.
- The optical unit has its foam cover attached.

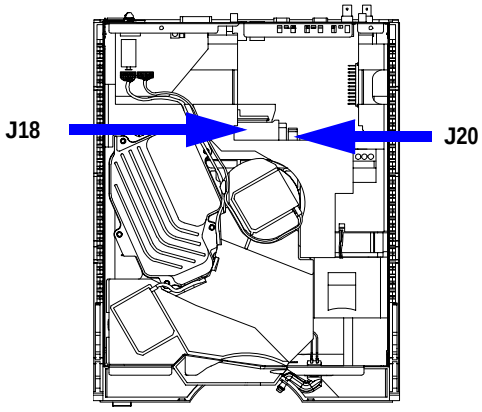
1 Place the optical unit in the instrument. Reconnect the grounding screw.



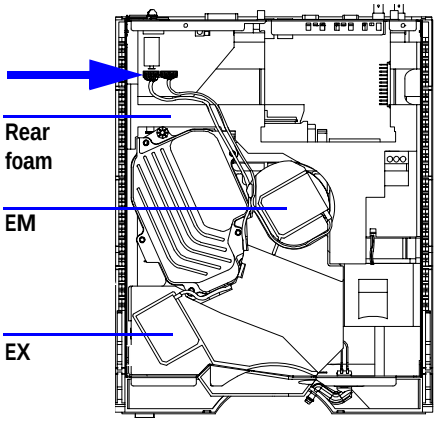
2 Reconnect the leak sensor cable (J3) using your left hand.



3 Reconnect the optical unit cable (J18) and the EM motor cable (J20).



4 Insert the rear foam and reconnect cables (EM monochromator) and (EX monochromator) to the detector main board (FLM).



Next Steps:

- Replace the covers as described in "Replacing the Foams and Covers" on page 254.
- Re-install the flow cell and the front panel
- Re-install the detector in the stack.
- Turn the lamp ON.
- Perform a wavelength verification to check the correct positioning of the replaced assemblies, as described in chapter "Diagram of Light Path" on page 138.

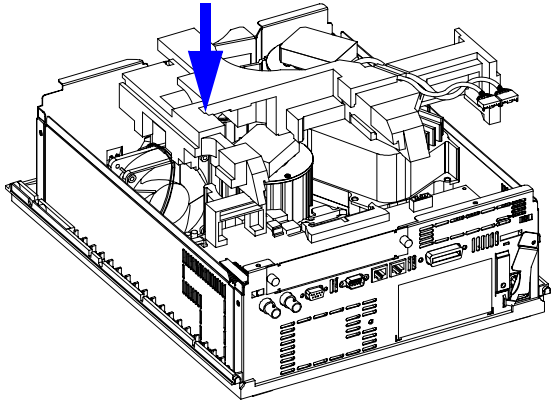
Replacing the Foams and Covers

NOTE If a new optical unit has been installed or assemblies within the optical unit have been replaced, then refer “Next Steps” on page 247.

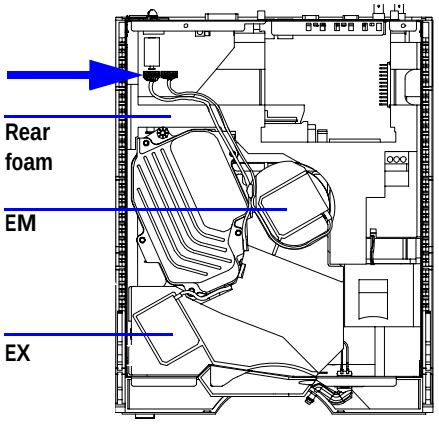
When required	When all repairs have been completed
Tools required	Screwdriver POZI 1 PT3
Prerequisites	The detector is open and other procedures have been carried out

NOTE The front cover (across the optical unit) should only be removed when required during a procedure. This will keep dust away from optical components.

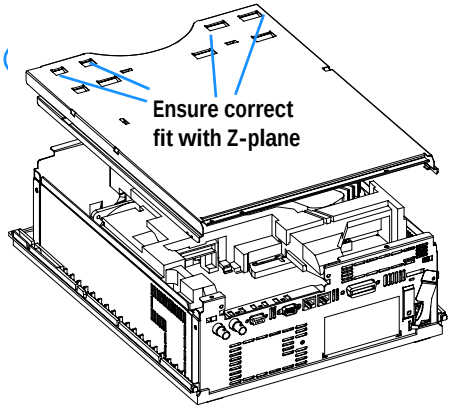
1 Carefully insert the rear foam.



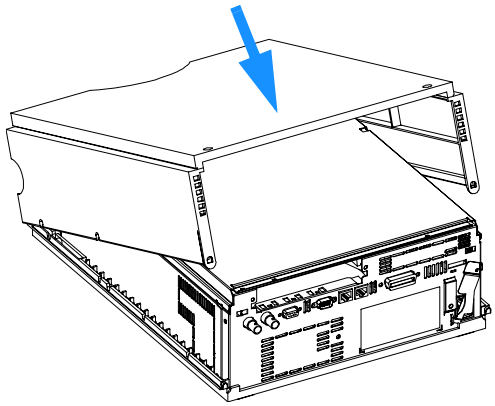
2 Route the cables towards the main board and reconnect to their connectors.



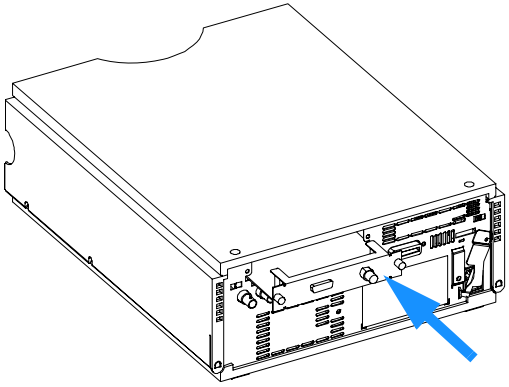
3 Slide the top plate towards the rear and fix the top plate screws.



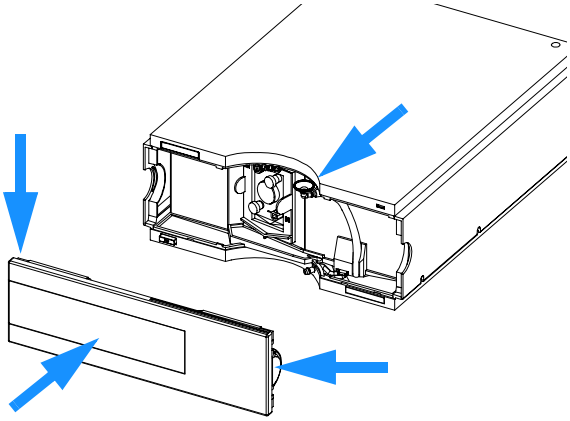
4 Replace the cover.



5 If required, insert the interface board and fix the screws.



6 Replace the waste funnel assmbly and the front panel.



Next Steps:

- Replace the detector into the stack and Reinstall the flow cell.
- Reconnect the power cable and turn on the detector.
- Turn the lamp ON.

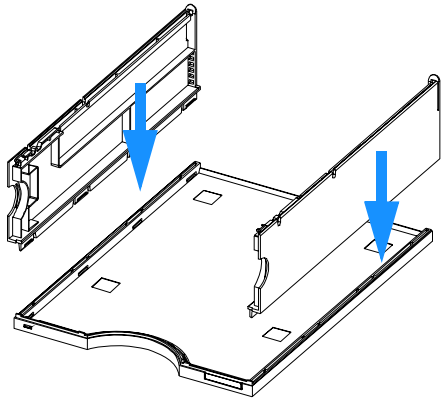
Assembling the Main Cover

When required	If cover is broken
Tools required	None
Parts required	Plastics kit 5062-8582 (includes base, top, left and right)

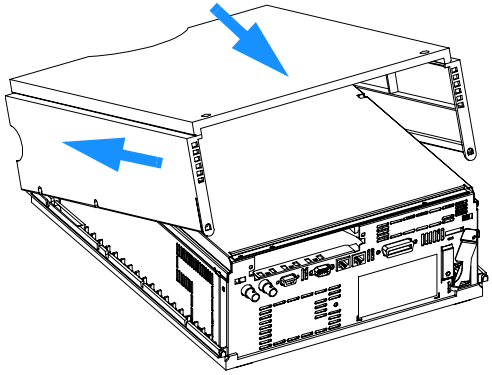
NOTE The plastics kit contains all parts, but it is not assembled.

WARNING **If you mistakenly insert the left or right side in the opposite position, you may not be able to remove that side from the top part.**

1 Place the top part on the bench and insert the left and right sides into the top part.



2 Replace the cover.



Next steps:

- Replace the detector in the stack and reconnect the cables and capillaries.
- Turn ON the detector.

11 Repairs

12
Parts and Materials for Maintenance

Overview of Maintenance Parts 260
Cuvette Kit 261
Spare Parts 262
Accessory Kit 263

This chapter provides information on parts for maintenance.

Overview of Maintenance Parts

Table 29 Maintenance Parts

Item	Description	Part Number
	Control Module G1323B or Instant Pilot G4208A	G1323-67001 G4208-67001
	Standard Flow Cell, 8 µl, 20 bar, inlet i.d./length 0.17 mm/80 mm, outlet i.d./length 0.25 mm/80 mm	G1321-60005
	Cuvette, 8 µl, 20 bar, see "Cuvette Kit" on page 261 inlet i.d./length 0.5 mm/80 mm, outlet i.d./length 0.5 mm/80 mm	G1321-60007
	Needle-Syringe	9301-0407
	Glass-Syringe	9301-1446
	Parts for wavelength calibration, see "Accessory Kit" on page 263	
	Front cover	5062-8592
	Leak funnel	5041-8388
	Leak funnel holder	5041-8389
	Clip	5041-8387
	Corrugated tubing, 120 mm lg, re-order 5 m	5062-2463
	Teflon Tubing flexible i.d. 0.8 mm (flow cell to waste)	5062-2462
	Cable CAN to Agilent 1200 Series modules (0.5 m)	5181-1516
	Cable CAN to Agilent 1200 Series modules (1 m)	5181-1519
	LAN Communication Interface Board (G1369A)	G1369-60001
	Cross-over network cable (shielded, 3 m long) for point to point connection	5023-0203
	Twisted pair network cable (shielded, 7 m long), for hub connections	5023-0202
	Analog cable (BNC to general purpose, spade lugs)	01046-60105
	Interface board BCD (BCD/external contacts)	G1351-68701

Cuvette Kit

Table 30 Cuvette Kit

Item	Description	Part Number
	FLD Cuvette Kit, 8 µl, 20 bar	G1321-60007
	includes	
	Tubing, flexible, 1 meter	
	SST Fitting, QTY=1	79814-22406
	SST front ferrule, QTY=1	0100-0043
	SST back ferrule, QTY=1	0100-0044
	PEEK fitting, QTY=1	0100-1516
	Needle-Syringe	9301-0407
	Glass-Syringe	9301-1446

Spare Parts

The spare parts listed below allow changes to the standard hardware configuration to adapt to specific application needs (as it was possible on the HP 1046A Fluorescence detector).

NOTE The installation of these parts may affect the performance of the detector and may not fulfill the instruments specifications.

Table 31 Spare Parts

Description	Part Number
Cutoff filter kit: 389 nm, 408 nm, 450 nm, 500 nm, 550 nm	5061-3327
Cutoff filter kit: 380 nm, 399 nm, 418 nm, 470 nm, 520 nm	5061-3328
Cutoff filter kit: 280 nm, 295 nm, 305 nm, 335 nm, 345 nm	5061-3329
Cutoff filter 370 nm	1000-0822
Photo-multiplier Tube (PMT) R928HA (185 to 900 nm)	contact Hamamatsu dealers
Photo-multiplier Tube (PMT) R3788HA (185 to 750 nm)	

Accessory Kit

This kit contains some accessories and tools needed for the installation and repair/calibration of the detector.

Table 32 Accessory Kit Parts

Item	Description	Part Number
	Accessory kit	G1321-68705
	includes	
	Corrugated tubing, 120 mm lg, re-order 5 m	5062-2463
1	Teflon Tubing flexible i.d. 0.8 mm (flow cell to waste), re-order 5 m	5062-2462
2	Fitting male PEEK, Qty=2	0100-1516
3	Capillary column – detector 380 mm lg, 0.17 i.d. includes items 4, 5 and 6 (not assembled)	G1315-87311
4	Ferrule front SST, qty=1	0100-0043
5	Ferrule back SST, qty=1	0100-0044
6	Fitting SST, qty=1	79814-22406
	Screwdriver hexagonal 4 mm, 100 mm long	5965-0027
	Screwdriver hexagonal 2.5 mm, 100 mm long	5965-0028
	Needle-Syringe	9301-0407
	Glass-Syringe	9301-1446
	Calibration Sample, Glycogen	5063-6597
	Sample filter, diameter=3 mm, pore size 0.45 µm, QYT=5	5061-3367 (pack of 100)
	Hex key set 1 – 5 mm	8710-0641
	Wrench open end 1/4 – 5/16 inch	8710-0510

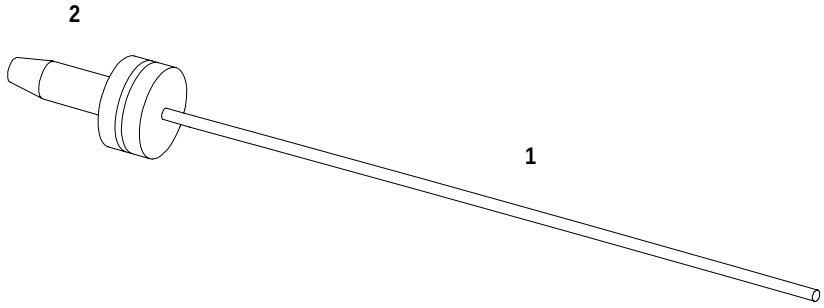


Figure 79 Waste Tubing Parts

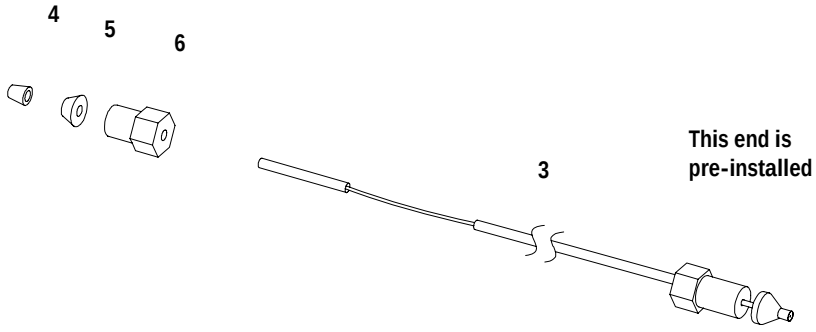


Figure 80 Inlet Capillary (Column-Detector) Parts

13
Parts for Repair

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Optical Unit Assembly 268
Lamp Cover Parts 271
Sheet Metal Kit 272
Plastic Parts 273
Foam Parts 274
Power and Status Light Pipes 275
Leak Parts 276

This chapter provides information on parts for repair.

Overview of Main Assemblies

Figure 81 shows the main assemblies and their locations.

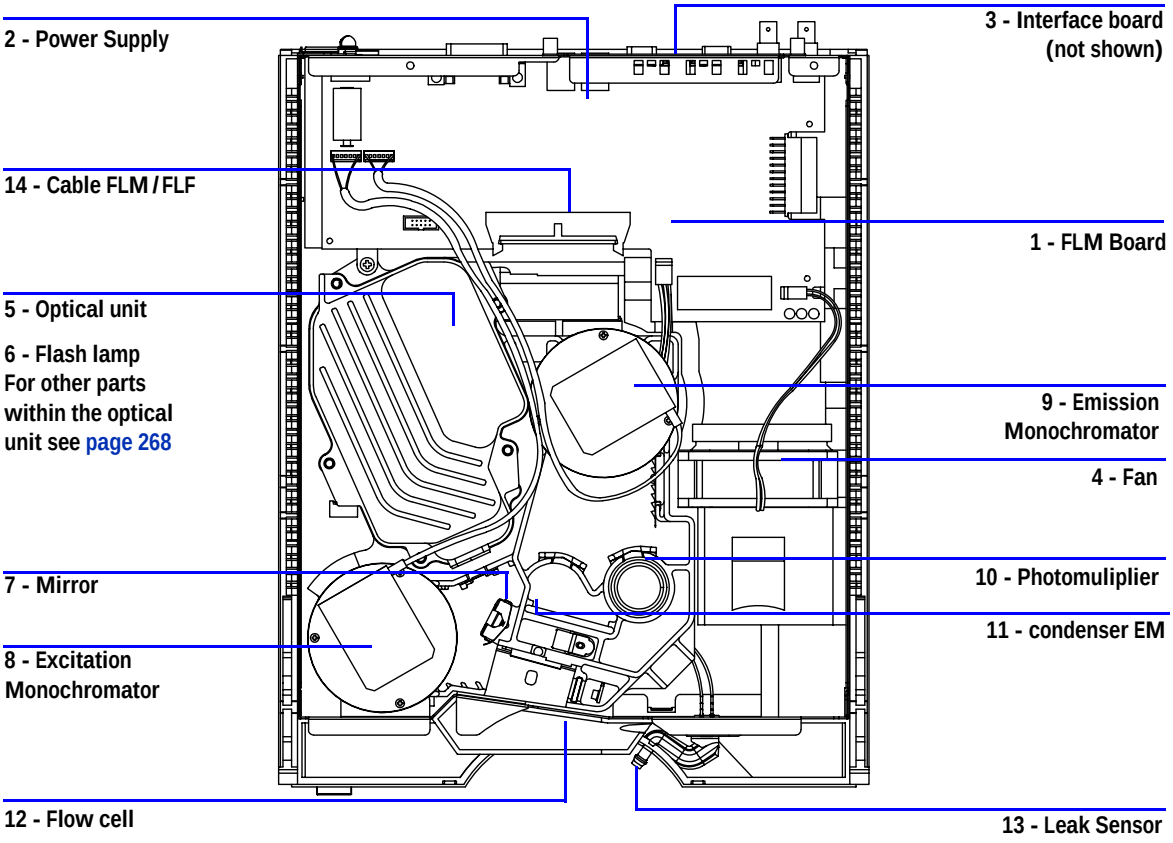

2 - Power Supply
3 - Interface board (not shown)
14 - Cable FLM / FLF
1 - FLM Board
5 - Optical unit
6 - Flash lamp
For other parts within the optical unit see page 268
9 - Emission Monochromator
4 - Fan
7 - Mirror
10 - Photomuliplier
11 - condenser EM
8 - Excitation Monochromator
12 - Flow cell
13 - Leak Sensor

Figure 81 Main Assemblies

Table 33 Main Assemblies

Item	Description	Part Number
1	Detector main board FLM (**new**)	G1321-66500
1	Detector main board FLM (**exchange assembly**)	G1321-69500
	Hexagonal nut for GPIB connector	0380-0643
	Hexagonal nut for RS-232C connector	1251-7788
	Nut for analog connector	2940-0256
	Washer for analog connector	2190-0699
	Cable CAN to Agilent 1200 Series modules (0.5 m)	5181-1516
2	Power supply for power and status light parts, see page 275	0950-2528
3	Interface board BCD (BCD/external contacts), optional, optional, see page 312	G1351-68701
3	LAN Communication Interface Board G1369A (LAN), optional, see page 314	G1369-60001
	Fuse for BCD board, 250 mA (total of 4 are on the board)	2110-0004
4	Fan assembly	3160-1017
5	Optical unit (**exchange assembly**), for additional optical unit parts, see page 268	G1321-69002
6	Xenon flash lamp	2140-0600
7	Mirror	1000-1000
8	Excitation Monochromator assembly (complete)	G1321-60003
9	Emission Monochromator assembly (complete)	G1321-60004
10	Photomuliplier tube assembly (PMT), see also page 262 for other types	1970-0201
11	Condenser EM, same as EX	1000-1124
12	Standard flow cell, 8 µl, 20 bar, see also page 268 for cell screws	G1321-60005
13	Leak sensor assembly and Leak handling parts, see page 276	5061-3356
14	Cable FLM - FLF board (comes with optical unit)	G1321-61600
	Front cover and plastic parts (housing), see page 273	
	Sheet metal parts, see page 272	
	Foam parts, see page 274	

Optical Unit Assembly

Table 34 Optical Unit Assembly

Item	Description	Part Number
	Optical unit (**new**), comes with foam part and CableFLM/FLF	G1321-66002
	Optical unit (**exchange assembly**), comes with foam part and CableFLM/FLF	G1321-69002
1	Flash Lamp Board (FLL), **includes** trigger pack	G1321-66512
2	Xenon flash lamp	2140-0600
3	Condenser Excitation	1000-1136
4	Slit (EX) 2x4	G1321-08103
5	Mirror	1000-1000
6	Excitation Monochromator assembly (complete)	G1321-60003
7	Emission Monochromator assembly (complete)	G1321-60004
8	Standard Flow Cell, 8 µl, 20 bar, inlet i.d./length 0.17 mm/80 mm, outlet i.d./length 0.25 mm/80 mm	G1321-60005
	Cuvette, 8 µl, 20 bar inlet i.d./length 0.5 mm/80 mm, outlet i.d./length 0.5 mm/80 mm	G1321-60007
	Screw for flow cell / cuvette	G1321-22400
9	Slit (EM) 2x8	G1321-08102
10	Photomuliplier tube assembly (PMT), see also page 236 for additional information	1970-0201
	Photo-multiplier Tube (PMT) R928HA (185 to 900 nm)	contact Hamamatsu dealers
	Photo-multiplier Tube (PMT) R3788HA (185 to 750 nm)	
11	Condenser Emission	1000-1124
12	Front-end Board (FLF) Rev D, underneath the optical (**new**) (requires firmware revision A.04.06 or above)	G1321-66531
12	Front-end Board (FLF) Rev D, underneath the optical (**exchange assembly**) (requires firmware revision A.04.06 or above)	G1321-69531

Table 34 Optical Unit Assembly (continued)

Item	Description	Part Number
13	Reference Diode Board (FLR)	G1321-66533
14	Diffuser plate (in front of the reference diode board FLR)	1000-1121
15	Cutoff Filter 295 nm, for other type see page 262	5062-8530
16	Carbon filter mat	01046-42701
17	Cable FLM - FLF board (comes with optical unit)	G1321-61600
	Foam Optics	G1321-40103
18	Cable chassis	G1321-61602
19	Screw M5 x 25 mm, for chassis cable	0515-2574
	Screw 2.5 mm hex, for monochromator, lamp pad, condenser #1, slits, etc.	0515-1052
	Screw M4 x 25 mm hex, for lamp housing	0515-2548
	Screw M4 x 8 mm, for FLF board	0515-2549

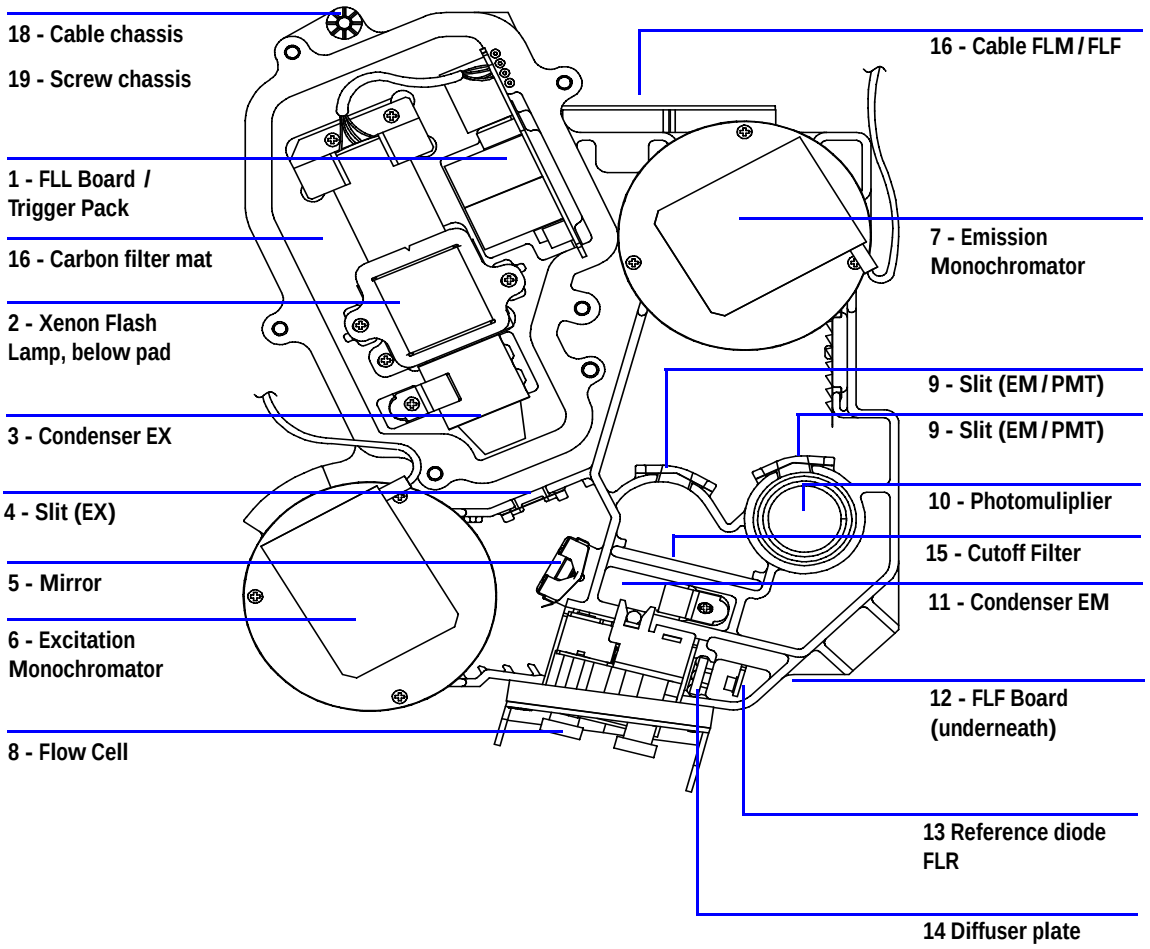

18 - Cable chassis
19 - Screw chassis
1 - FLL Board / Trigger Pack
16 - Carbon filter mat
2 - Xenon Flash Lamp, below pad
3 - Condenser EX
4 - Slit (EX)
5 - Mirror
6 - Excitation Monochromator
8 - Flow Cell
16 - Cable FLM / FLF
7 - Emission Monochromator
9 - Slit (EM / PMT)
9 - Slit (EM / PMT)
10 - Photomuliplier
15 - Cutoff Filter
11 - Condenser EM
12 - FLF Board (underneath)
13 Reference diode FLR
14 Diffuser plate

Figure 82 Optical Unit Parts

Lamp Cover Parts

Table 35 Lamp Cover Parts

Item	Description	Part Number
1	Plate	G1321-08100
2	Screw 2.5 mm hex	0515-1052
3	Quartz Window	1000-0999

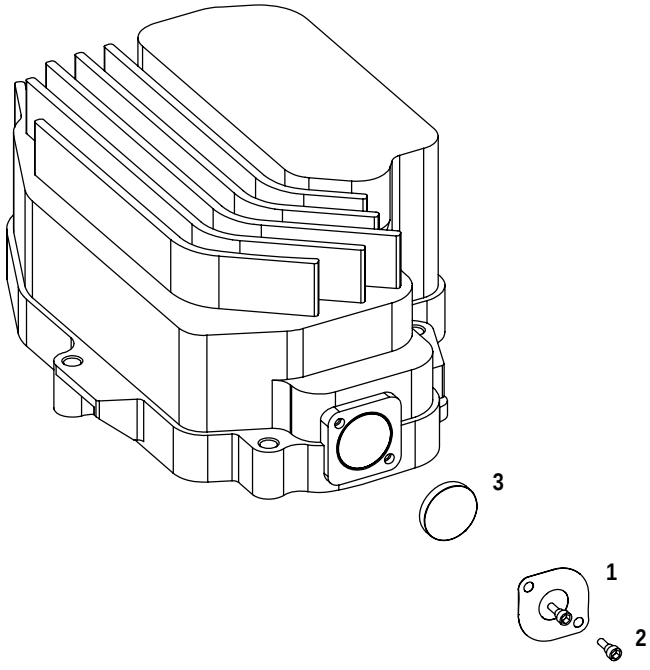


Figure 83 Lamp Cover Parts

Sheet Metal Kit

Table 36 Sheet Metal Kit Parts

Item	Description	Part Number
1	Sheet metal kit includes case and top cover	G1321-68701
2	Screws M3 for cover and flow cell door	5022-2112

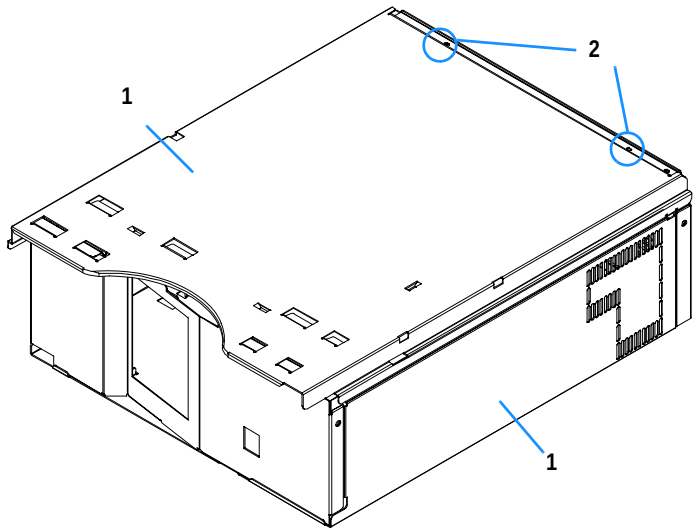


Figure 84 Sheet Metal Kit Parts

Plastic Parts

Table 37 Plastics Parts

Item	Description	Part Number
1	Front cover	5065-9982
2	Plastics, includes base, sides and top	5065-9985
3	Name plate Agilent 1200 Series	5042-8901

NOTE

For correct assembling of the top and sides, see "Assembling the Main Cover" on page 256.

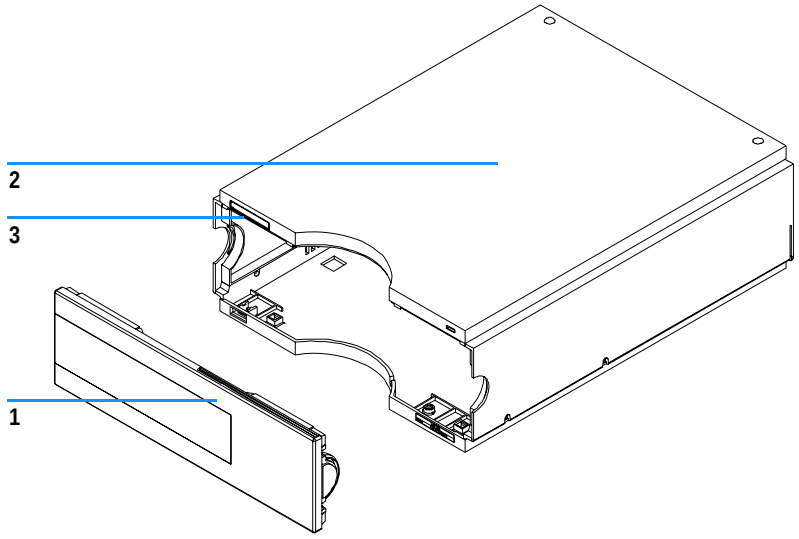


Figure 85 Plastic Parts

Foam Parts

Table 38 Foam Parts

Item	Description	Part Number
1, 2	EPP foam kit, includes bottom and top rear	G1321-68702
3	Foam Pads (located underneath the optical), set of two	G1321-68700
4	Guides for interface board	5041-8395

NOTE

Do not order the individual part numbers imprinted on the foam.

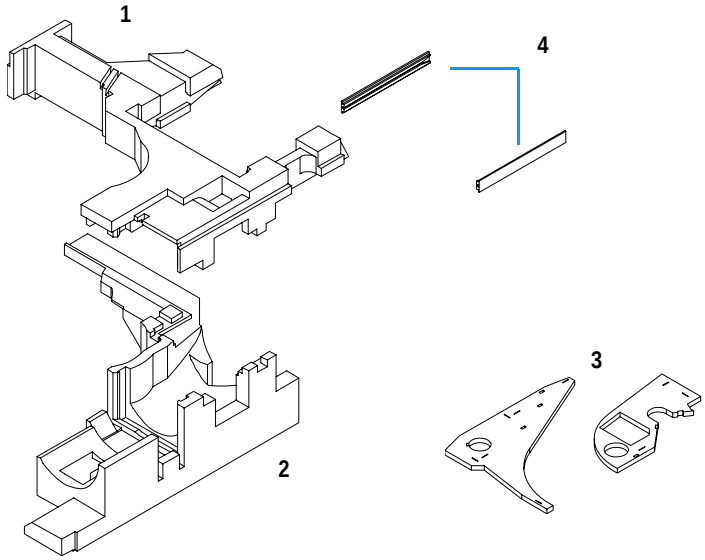


Figure 86 Foam Parts

Power and Status Light Pipes

Table 39 Power and Status Light Pipes

Item	Description	Part Number
	Power supply assembly	0950-2528
	Screw M4 x 0.7, 8 mm lg, to fix power supply at rear panel	0515-0910
	Washer	2190-0409
1	Power light pipe	5041-8382
2	Status light pipe	5041-8384
3	Power switch button	5041-8381
4	Coupler for power supply actuator	5041-8383

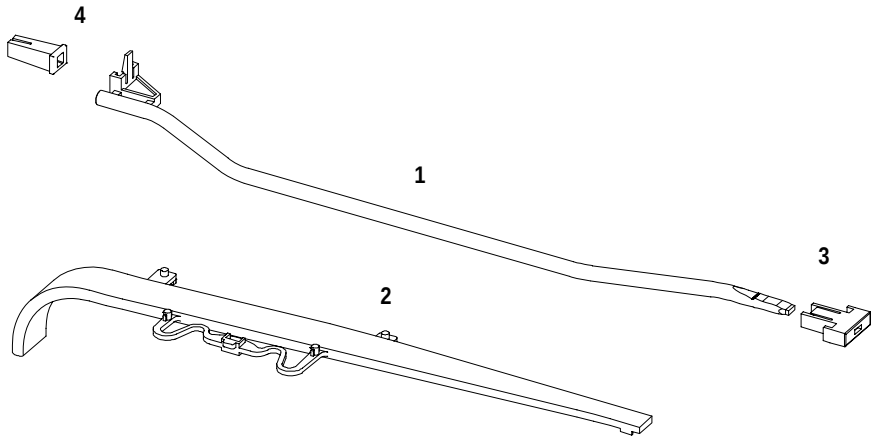


Figure 87 Power and Status Light Pipe

Leak Parts

Table 40 Leak Parts

Item	Description	Part Number
1	Leak sensor assembly	5061-3356
2	Leak pan	G1321-40511
3	Leak funnel	5041-8388
4	Leak funnel holder	5041-8389
5	Corrugated tubing, 120 mm lg, re-order 5 m	5062-2463
6	Corrugated tubing, 1200 mm lg, re-order 5 m	5062-2463

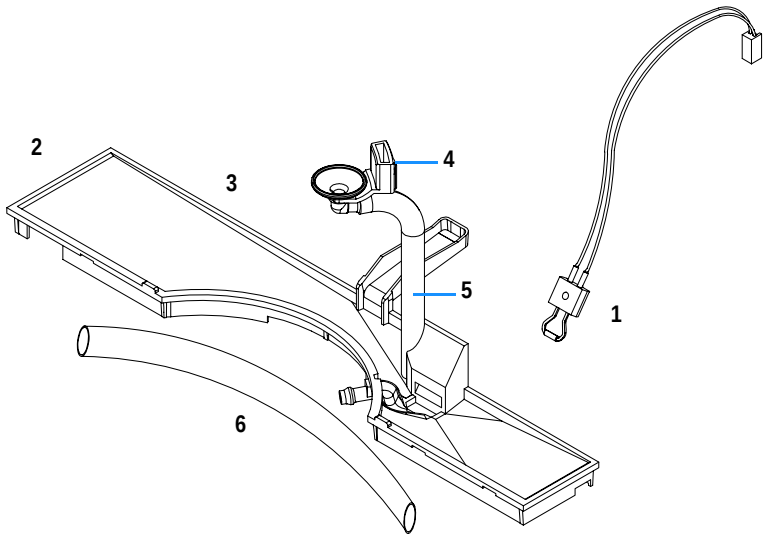


Figure 88 Leak Parts

14 Cables

Cable Overview 278
Analog Cables 280
Remote Cables 283
BCD Cables 288
Auxiliary Cable 290
CAN Cable 291
External Contact Cable 292
RS-232 Cable Kit 293
LAN Cables 294

This chapter provides information on cables used with the 1200 series of HPLC modules.

Cable Overview

WARNING **Never use cables other than the ones supplied by Agilent Technologies to ensure proper functionality and compliance with safety or EMC regulations.**

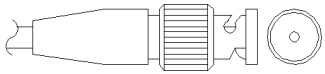
Table 41 Cables Overview

Type	Description	Part Number
Analog cables	3390/2/3 integrators	01040-60101
	3394/6 integrators	35900-60750
	35900A A/D converter	35900-60750
	General purpose (spade lugs)	01046-60105
Remote cables	3390 integrator	01046-60203
	3392/3 integrators	01046-60206
	3394 integrator	01046-60210
	3396A (Series I) integrator	03394-60600
	3396 Series II / 3395A integrator, see page 285	
	3396 Series III / 3395B integrator	03396-61010
	Agilent 1200/ 1100 / 1050 modules / 1046A FLD	5061-3378
	1046A FLD	5061-3378
	35900A A/D converter	5061-3378
	1090 liquid chromatographs	01046-60202
	Signal distribution module	01046-60202

Table 41 Cables Overview (continued)

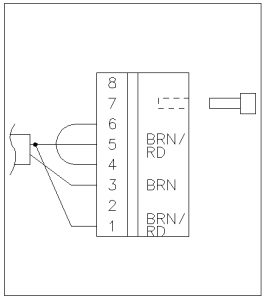
Type	Description	Part Number
BCD cables	3396 integrator	03396-60560
	General purpose (spade Lugs)	G1351-81600
Auxiliary	Agilent 1200 Series vacuum degasser	G1322-61600
CAN cables	Agilent 1200 module to module, 0.5 m	5181-1516
	Agilent 1200 module to module, 1 m	5181-1519
	Agilent 1200 module to Control Module G1323B	G1323-81600
External contacts	Agilent 1200 Series interface board to general purpose	G1103-61611
GPIB cable	Agilent 1200 module to Agilent ChemStation, 1 m	10833A
	Agilent 1200 module to Agilent ChemStation, 2 m	10833B
RS-232 cable	Agilent 1200 module to a computer This kit contains a 9-pin female to 9-pin female Null Modem (printer) cable and one adapter.	34398A
LAN cable	Cross-over network cable (shielded, 3 m long), (for point to point connection)	5023-0203
	Twisted pair network cable (shielded, 7 m long) (for hub connections)	5023-0202

Analog Cables

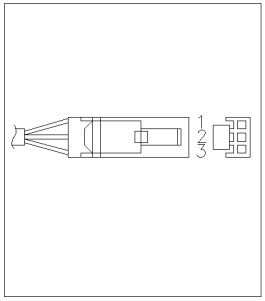


One end of these cables provides a BNC connector to be connected to Agilent 1200 Series modules. The other end depends on the instrument to which connection is being made.

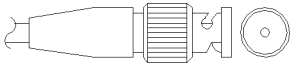
Agilent 1200 to 3390 / 2 / 3 Integrators

Connector 01040-60101	Pin 3390 / 2 / 3	Pin Agilent 1200	Signal Name
	1	Shield	Ground
	2		Not connected
	3	Center	Signal +
	4		Connected to pin 6
	5	Shield	Analog -
	6		Connected to pin 4
	7		Key
	8		Not connected

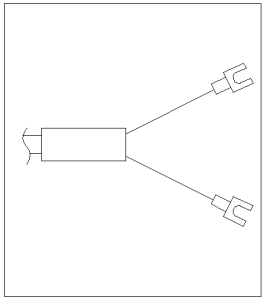
Agilent 1200 to 3394/6 Integrators

Connector 35900-60750	Pin 3394/6	Pin Agilent 1200	Signal Name
	1		Not connected
	2	Shield	Analog -
	3	Center	Analog +

Agilent 1200 to BNC Connector

Connector 8120-1840	Pin BNC	Pin Agilent 1200	Signal Name
	Shield	Shield	Analog -
	Center	Center	Analog +

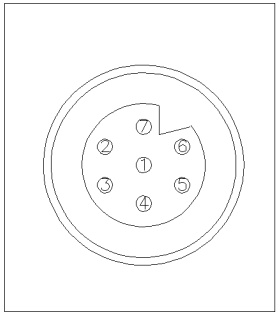
Agilent 1200 to General Purpose

Connector 01046-60105	Pin 3394 / 6	Pin Agilent 1200	Signal Name
	1		Not connected
	2	Black	Analog -
	3	Red	Analog +

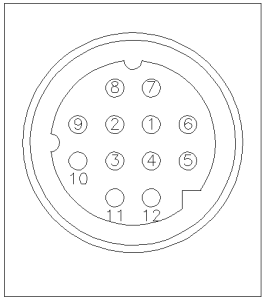
Remote Cables

One end of these cables provides a Agilent Technologies APG (Analytical Products Group) remote connector to be connected to Agilent 1200 Series modules. The other end depends on the instrument to be connected to.

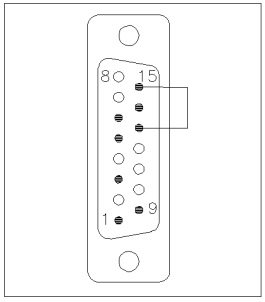
Agilent 1200 to 3390 Integrators

Connector 01046-60203	Pin 3390	Pin Agilent 1200	Signal Name	Active (TTL)
	2	1 - White	Digital ground	
	NC	2 - Brown	Prepare run	Low
	7	3 - Gray	Start	Low
	NC	4 - Blue	Shut down	Low
	NC	5 - Pink	Not connected	
	NC	6 - Yellow	Power on	High
	NC	7 - Red	Ready	High
	NC	8 - Green	Stop	Low
	NC	9 - Black	Start request	Low

Agilent 1200 to 3392 / 3 Integrators

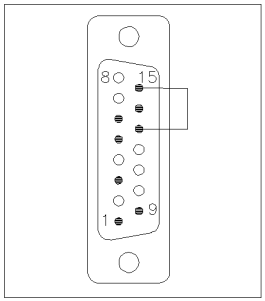
Connector 01046-60206	Pin 3392 / 3	Pin Agilent 1200	Signal Name	Active (TTL)
 4 - Key	3	1 - White	Digital ground	
	NC	2 - Brown	Prepare run	Low
	11	3 - Gray	Start	Low
	NC	4 - Blue	Shut down	Low
	NC	5 - Pink	Not connected	
	NC	6 - Yellow	Power on	High
	9	7 - Red	Ready	High
	1	8 - Green	Stop	Low
	NC	9 - Black	Start request	Low

Agilent 1200 to 3394 Integrators

Connector 01046-60210	Pin 3394	Pin Agilent 1200	Signal Name	Active (TTL)
	9	1 - White	Digital ground	
	NC	2 - Brown	Prepare run	Low
	3	3 - Gray	Start	Low
	NC	4 - Blue	Shut down	Low
	NC	5 - Pink	Not connected	
	NC	6 - Yellow	Power on	High
	5,14	7 - Red	Ready	High
	6	8 - Green	Stop	Low
	1	9 - Black	Start request	Low
	13, 15		Not connected	

NOTE START and STOP are connected via diodes to pin 3 of the 3394 connector.

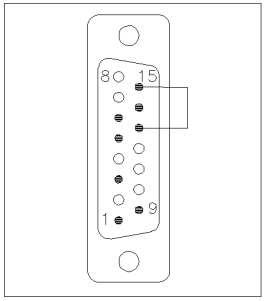
Agilent 1200 to 3396A Integrators

Connector 03394-60600	Pin 3394	Pin Agilent 1200	Signal Name	Active (TTL)
	9	1 - White	Digital ground	
	NC	2 - Brown	Prepare run	Low
	3	3 - Gray	Start	Low
	NC	4 - Blue	Shut down	Low
	NC	5 - Pink	Not connected	
	NC	6 - Yellow	Power on	High
	5,14	7 - Red	Ready	High
	1	8 - Green	Stop	Low
	NC	9 - Black	Start request	Low
	13, 15		Not connected	

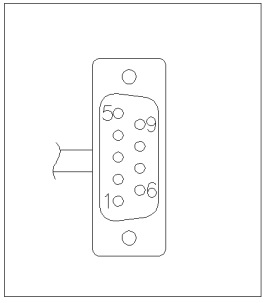
Agilent 1200 to 3396 Series II / 3395A Integrators

Use the cable 03394-60600 and cut pin #5 on the integrator side. Otherwise the integrator prints START; not ready.

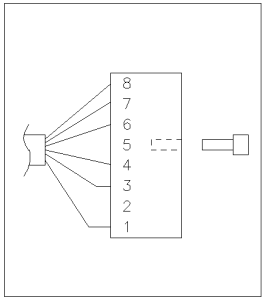
Agilent 1200 to 3396 Series III / 3395B Integrators

Connector 03396-61010	Pin 33XX	Pin Agilent 1200	Signal Name	Active (TTL)
	9	1 - White	Digital ground	
	NC	2 - Brown	Prepare run	Low
	3	3 - Gray	Start	Low
	NC	4 - Blue	Shut down	Low
	NC	5 - Pink	Not connected	
	NC	6 - Yellow	Power on	High
	14	7 - Red	Ready	High
	4	8 - Green	Stop	Low
	NC	9 - Black	Start request	Low
	13, 15		Not connected	

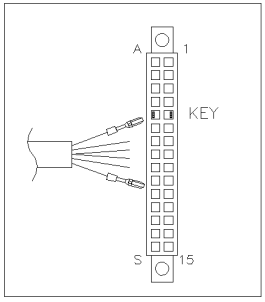
Agilent 1200 to HP 1050, HP 1046A or Agilent 35900 A / D Converters

Connector 5061-3378	Pin HP 1050 / ...	Pin Agilent 1200	Signal Name	Active (TTL)
	1 - White	1 - White	Digital ground	
	2 - Brown	2 - Brown	Prepare run	Low
	3 - Gray	3 - Gray	Start	Low
	4 - Blue	4 - Blue	Shut down	Low
	5 - Pink	5 - Pink	Not connected	
	6 - Yellow	6 - Yellow	Power on	High
	7 - Red	7 - Red	Ready	High
	8 - Green	8 - Green	Stop	Low
	9 - Black	9 - Black	Start request	Low

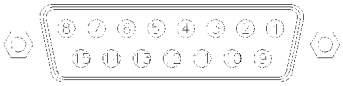
Agilent 1200 to HP 1090 LC or Signal Distribution Module

Connector 01046-60202	Pin HP 1090	Pin Agilent 1200	Signal Name	Active (TTL)
	1	1 - White	Digital ground	
	NC	2 - Brown	Prepare run	Low
	4	3 - Gray	Start	Low
	7	4 - Blue	Shut down	Low
	8	5 - Pink	Not connected	
	NC	6 - Yellow	Power on	High
	3	7 - Red	Ready	High
5 - Key	6	8 - Green	Stop	Low
	NC	9 - Black	Start request	Low

Agilent 1200 to General Purpose

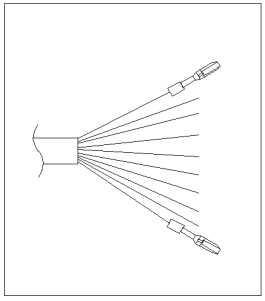
Connector 01046-60201	Pin Universal	Pin Agilent 1200	Signal Name	Active (TTL)
		1 - White	Digital ground	
		2 - Brown	Prepare run	Low
		3 - Gray	Start	Low
		4 - Blue	Shut down	Low
		5 - Pink	Not connected	
		6 - Yellow	Power on	High
		7 - Red	Ready	High
		8 - Green	Stop	Low
		9 - Black	Start request	Low

BCD Cables

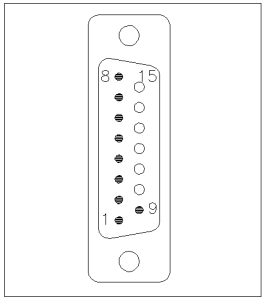


One end of these cables provides a 15-pin BCD connector to be connected to the Agilent 1200 Series modules. The other end depends on the instrument to be connected to

Agilent 1200 to General Purpose

Connector G1351-81600	Wire Color	Pin Agilent 1200	Signal Name	BCD Digit
	Green	1	BCD 5	20
	Violet	2	BCD 7	80
	Blue	3	BCD 6	40
	Yellow	4	BCD 4	10
	Black	5	BCD 0	1
	Orange	6	BCD 3	8
	Red	7	BCD 2	4
	Brown	8	BCD 1	2
	Gray	9	Digital ground	Gray
	Gray/pink	10	BCD 11	800
	Red/blue	11	BCD 10	400
	White/green	12	BCD 9	200
	Brown/green	13	BCD 8	100
	not connected	14		
	not connected	15	+5 V	Low

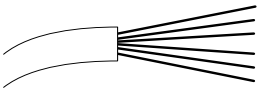
Agilent 1200 to 3396 Integrators

Connector 03396-60560	Pin 3392 / 3	Pin Agilent 1200	Signal Name	BCD Digit
	1	1	BCD 5	20
	2	2	BCD 7	80
	3	3	BCD 6	40
	4	4	BCD 4	10
	5	5	BCD0	1
	6	6	BCD 3	8
	7	7	BCD 2	4
	8	8	BCD 1	2
	9	9	Digital ground	
	NC	15	+ 5 V	Low

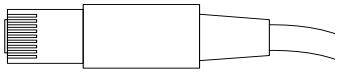
Auxiliary Cable

One end of this cable provides a modular plug to be connected to the Agilent 1200 Series vacuum degasser. The other end is for general purpose.

Agilent 1200 Series Degasser to general purposes

Connector G1322-61600	Color	Pin Agilent 1200	Signal Name
	White	1	Ground
	Brown	2	Pressure signal
	Green	3	
	Yellow	4	
	Grey	5	DC + 5 V IN
	Pink	6	Vent

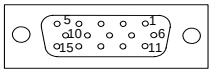
CAN Cable



Both ends of this cable provide a modular plug to be connected to Agilent 1200 Series module's CAN-bus connectors.

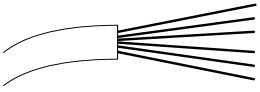
Agilent 1200 module to module, 0.5 m	5181-1516
Agilent 1200 module to module, 1 m	5181-1519
Agilent 1200 module to Control Module G1323B	G1323-81600

External Contact Cable



One end of this cable provides a 15-pin plug to be connected to Agilent 1200 Series module's interface board. The other end is for general purpose.

Agilent 1200 Series Interface Board to general purposes

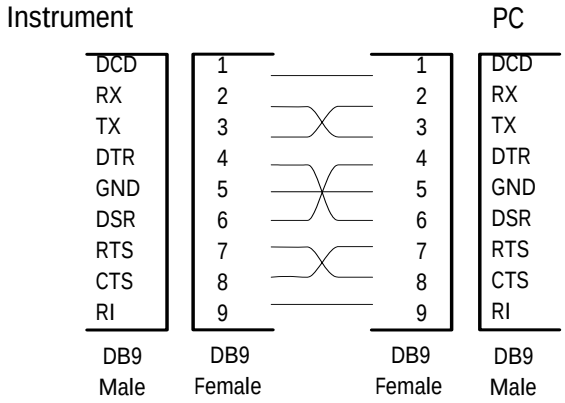
Connector G1103-61611	Color	Pin Agilent 1200	Signal Name
	White	1	EXT 1
	Brown	2	EXT 1
	Green	3	EXT 2
	Yellow	4	EXT 2
	Grey	5	EXT 3
	Pink	6	EXT 3
	Blue	7	EXT 4
	Red	8	EXT 4
	Black	9	Not connected
	Violet	10	Not connected
	Grey/pink	11	Not connected
	Red/blue	12	Not connected
	White/green	13	Not connected
	Brown/green	14	Not connected
	White/yellow	15	Not connected

RS-232 Cable Kit

This kit contains a 9-pin female to 9-pin female Null Modem (printer) cable and one adapter. Use the cable and adapter to connect Aligent Technologies instruments with 9-pin male RS-232 connectors to most PCs or printers.

Agilent 1200 module to PC

RS-232 Cable Kit 34398As



LAN Cables

Recommended Cables

Table 42

Description	Part number
Cross-over network cable (shielded, 3 m long), (for point to point connection)	5023-0203
Twisted pair network cable (shielded, 7 m long), (for hub connections)	5023-0202

15
Hardware Information

Detector Main Board (FLM) 296
Detector Frontend Board (FLF) 298
Detector Lamp Supply Board (FLL) 303
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Interfaces 315
Setting the 8-bit Configuration Switch 320
The Main Power Supply Assembly 325

This chapter describes the detector in more detail on hardware and electronics.

Detector Main Board (FLM)

This board controls all information and activities of all assemblies within the detector. Through interfaces (CAN, GPIB, RS-232C or LAN) connected to the user interface, the operator enters parameters, changes modes and controls the detector.

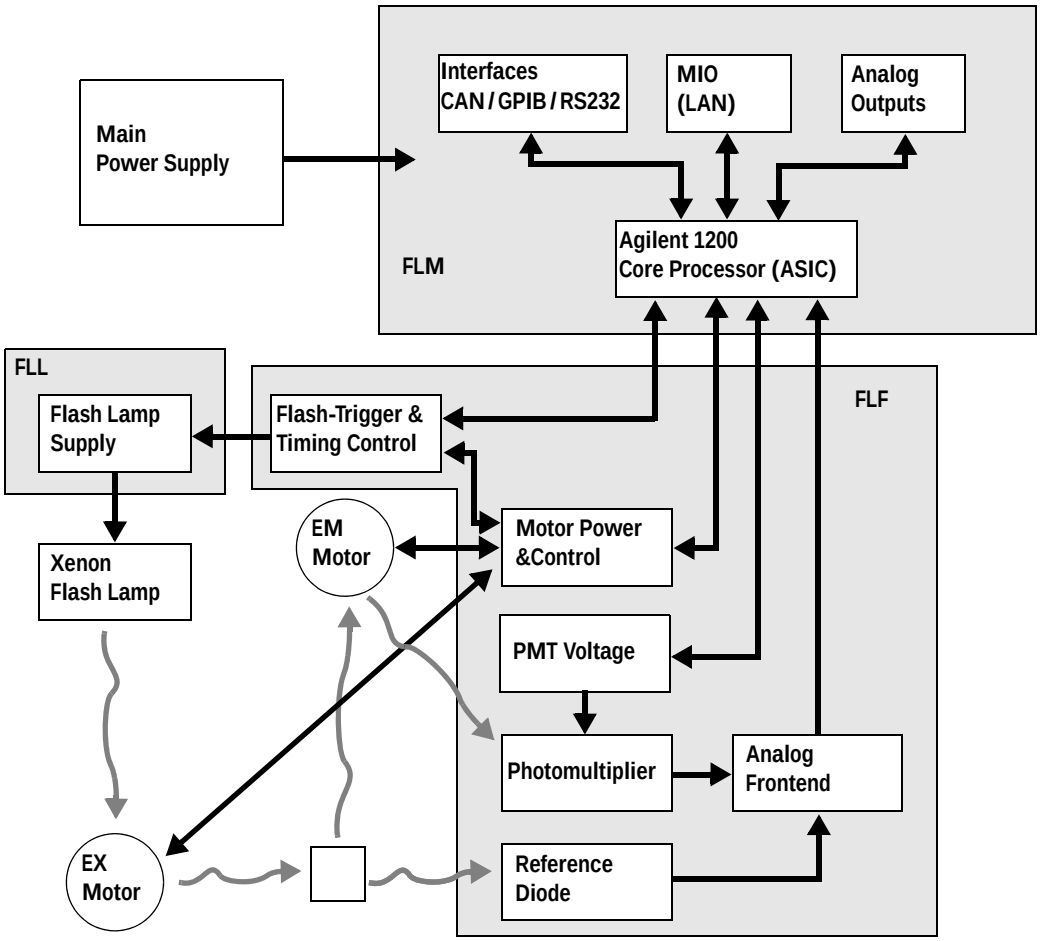

Figure 89 Detector Electronic Overview

Excitation / Emission Monochromator Motor Control

A chopped motor driver IC provides the signal to run the excitation/emission monochromator motor in positioning or rotation mode.

Encoder Signal Acquisition

Each monochromator includes an optical encoder to get the actual position of the device. The two encoder signals are amplified and prepared for A/D-conversion.

Fan Drive

The operation of the fan is controlled by the main processor and runs with constant revolution. The fan produces a sense signal which is derived from the revolution. This sense signal is used for diagnostics.

On-board Battery

An on-board lithium battery buffers the electronic memory when the module is turned OFF.

For safety information on lithium batteries see “Lithium Batteries Information” on page 331.

Analog Outputs

There are two independent analog outputs (0...1 V full scale). Digital data from the ASIC is converted into a pulse-width modulated signal (PWM). The PWM signal is then fed to an amplitude modulator, which precisely turns the reference signal on and off. This signal then passes to a low-pass filter section.

Interfaces

For detailed information on interfaces, see “Interfaces” on page 315.

Detector Frontend Board (FLF)

Data Acquisition

The FLF board provides circuitry to measure three different physical effects:

- Fluorescence mode: peak value after flash
- Phosphorescence mode: integrated value after flash
- Chemoluminescence mode: amplified signal without flash operation

In parallel to the fluorescence signal the reference signal is acquired to get a compensation of lamp drift and noise effects. Both signals are amplified and buffered and routed to the ADC input multiplexer.

The 16-bit A/D-converter performs a conversion of the selected signal and additionally of the corresponding reference signal and several other signals:

- monochromator positions
- board temperature
- leak sensor status

Then the digital data are polled by the FPGA on the FLM main board to be available for calculations.

Flash Lamp Power Supply

The flash lamp is operated with discharge voltages of 500 V or 1250 V. The electronics design provides the voltages using a regulating pulse width modulator to switch a 24 V rail with a chopping frequency of 40 kHz to a transformer. The resulting high voltage is rectified and fed back to the error amplifiers which generate a control feedback signal for the regulator to increase or decrease the duty cycle of the output signal. An additional softstart-circuitry and a limitation of the maximum voltage ramp-up reduces the resulting noise emission of the power supply. The complete flash voltage supply is isolated by optocouplers to avoid crosstalk to other circuits by the large switching currents.

Flash Trigger Voltage Supply

The flash lamp trigger assembly includes an ignition transformer which needs a 160 V pulse to generate a short high voltage pulse (~ 10 kV) for a reliable lamp ignition. A second lamp power supply transformer output is used to generate this trigger voltage at both operating conditions.

Igniting the Flash Lamp

The flash lamp is a plasma discharge lamp with an energy-controlled intensity operated at 74 Hz or 296 Hz. The applied voltage charges a capacitor array and forms a controlled discharge voltage difference between lamp anode and cathode. Then an additional ignition electrode is driven with a high ignition voltage pulse to build up a small ionized channel from the ignition electrode to the cathode. If this ionization is existent, the voltage difference between anode and cathode leads to a flash discharge within a very short time (1 µs). After the flash the system pauses the regulator to force the ionized path to break down rapidly before charging the capacitors for the next cycle.

Flash Lamp Overcurrent Protection

A special firmware-controlled diagnostic circuitry shuts down the regulator when the charge current exceeds a critical value. This may happen when the output voltage lines are shorted. When an overcurrent condition is found, the circuit stops the voltage regulator within the time between two flashes, and the firmware reports an error signal when a restart of the regulator fails again.

Flash Voltage Safety Switch

A safety switch stops the voltage regulator when the lamp cover is removed to avoid high voltages on board when electronic parts are accessible.

Photomultiplier Tube Voltage Supply

The photomultiplier tube needs an adjustable working voltage between -240 V and -820 V for correct operation. The PMT gains are realized by variable operating voltage of the device. A regulated pulse width modulator is used to generate the high voltage by switching the 24 V rail to a transformer. The feedback loop has a D/A-converter-controlled base voltage to set the desired output voltage. The PMT output delivers a current signal which is proportional to the detected light and to the actual working voltage.

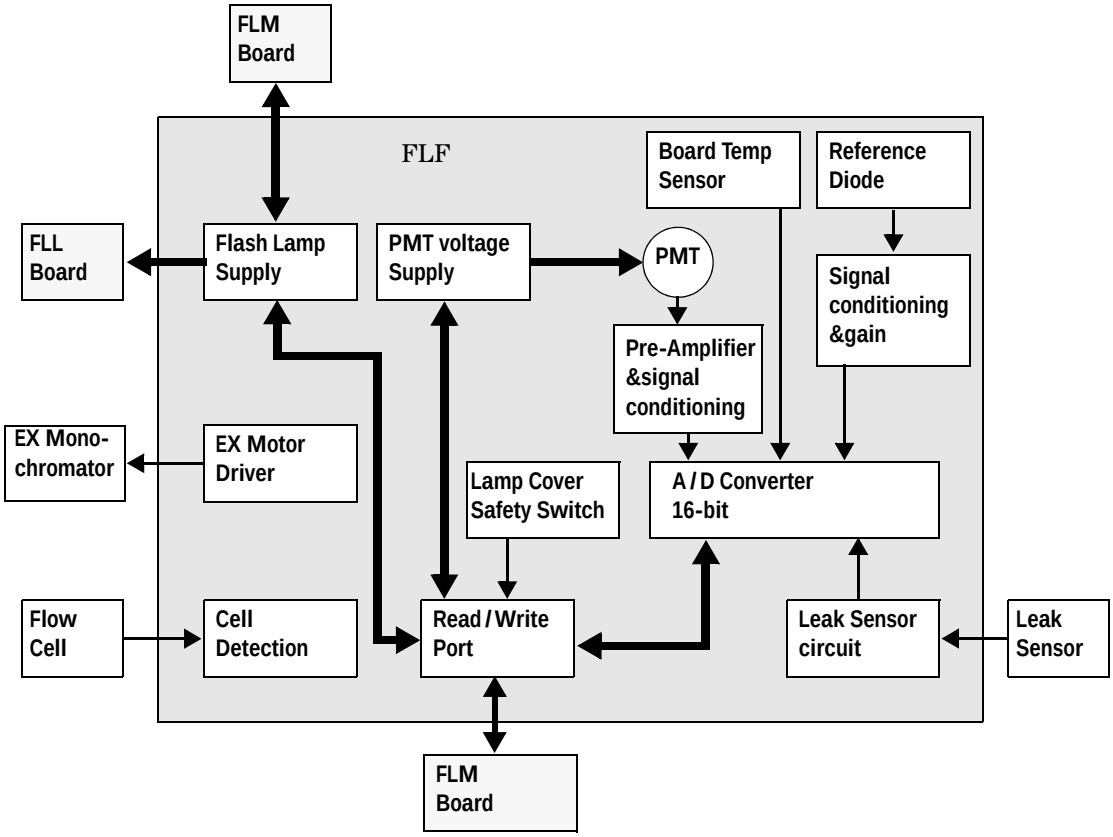

Figure 90 Block diagram FLF

Reference System

A reference photodiode is located behind the cell and monitors the incoming radiation through a diffusor window. The data are used to compensate the fluorescence data for drift effects. The reference path provides multiple gain stages which are set automatically according to the actual intensity.

Cell Detection

The system detects if a cell is inserted and switches off lamp operation, PMT high voltage and monochromator rotation if no cell is present to prevent injury (by UV radiation, high voltage or rotating parts) to the user.

Emission Monochromator Motor Control

A chopped motor driver IC provides the signal to run the excitation monochromator motor in positioning or rotation mode.

Leak Sensor

A leak sensor is mounted in the instrument's front chassis to detect leakage of the cell assy. In case of a leakage, the complete Agilent 1200 System is shut down to prevent system damage.

This block consists of a PTC for the leak detection and a NTC for the ambient temperature compensation. A leak would cool down the PTC and this change in resistance would generate a leak signal.

Digital Interface

The control and data lines from and to the Fluorescence Detector Main Board (FLM) are buffered by Read- and Write ports. The signals from the main board allow setting of the signal multiplexers, reference gain stages, lamp current and to start the initial self-calibration of the A/D-converter. The port to the main board drives the A/D-converter data and several control lines like cell detection, overcurrent detection and lamp cover safety switch position.

The signals driving the lamp are derived from the FPGA on the Main Board and have separate connections. This allows the lamp timing control without processor interaction and guarantees a realtime control of the lamp operation.

How to retrieve the FLF board revision

Agilent ChemStation

The FLF board revision can be retrieved with the following ChemStation command:

```
PRINT SENDMODULE$(LFLD,"FLFR?")
```

The reply will be: RA 0000 FLFR "AAAA" for revsion A boards.

Control Module (G1323B)

1. Select **FLD TESTS**
2. Press **m.m** (m-dot-m)
3. Select **Command**
4. Type: `FLFR?`
5. Press **Execute**.

NOTE

Firmware revisions below A.03.70 do not know this command (RE 0503).

Detector Lamp Supply Board (FLL)

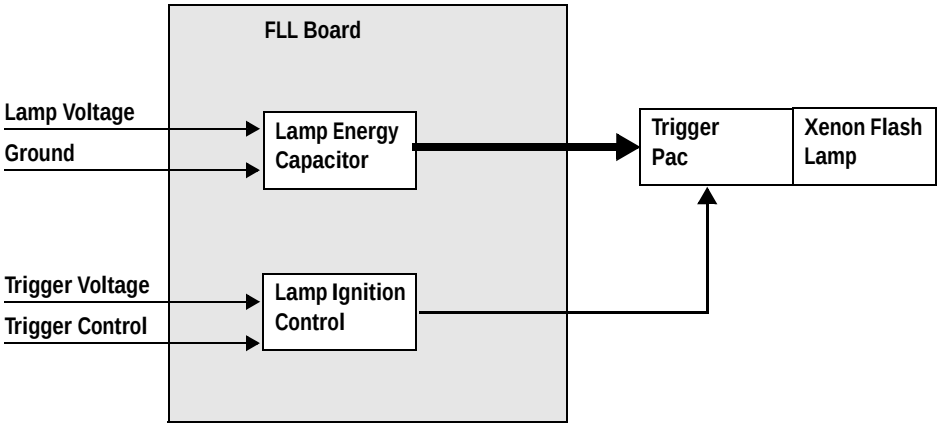

Figure 91 Block Diagram FLL Board

Lamp Energy Capacitors

The lamp high voltage circuit of the FLF board is used to charge the 4 energy capacitors of the FLL board. The energy per flash depends on the applied charging voltage ($1/2*C*U^2$). A power diode freewheeling circuit prevents the backflowing current from generating negative lamp discharge currents which may damage the flash lamp.

Lamp Ignition Control

An ignition voltage is switched by a thyristor to the TriggerPac ignition transformer to built a small ionized channel (refer "Igniting the Flash Lamp" on page 299). A freewheeling diode forces the thyristor to switch OFF again to prevent lamp damage.

Firmware Description

The firmware of the instrument consists of two independent sections:

- a non-instrument specific section, called **resident system**,
- an instrument specific section, called **main system**.

Resident System

This resident section of the firmware is identical for all Agilent 1200 Series modules. Its properties are:

- the complete communication capabilities (GPIB, CAN, LAN and RS-232C),
- memory management,
- ability to update the firmware of the 'main system'.

Main System

Its properties are:

- the complete communication capabilities (GPIB, CAN, LAN and RS-232C),
- memory management,
- ability to update the firmware of the resident system.

In addition the main system comprises the instrument functions that are divided into common functions like

- run synchronization through APG remote
- error handling,
- diagnostic functions,
- or module specific functions like:
  - internal events such as lamp control, filter movements,
  - raw data collection and conversion to absorbance (see "Raw Data Conversion to Fluorescence (LU) Units" on page 306.

Firmware Updates

Firmware updates can be done using your user interface:

- instant pilot G4208A with files from a USB-memory stick, or
- handheld Control Module G1323B with files from a PC-card, or
- a PC Firmware Update Tool with files from hard disk or CD-ROM.

The file naming conventions are:

1321A_A602_zz.dlb, where

xxxxx is the product number, e.g. 1321A for the G1321A FLD), and
vvvv is the revision number, for example A602 is revision A.06.02, and
zz is the build number of the firmware.

For instructions refer to your user interface.

NOTE

Update of main system can be done in the resident system only.

Update of the resident system can be done in the main system only.

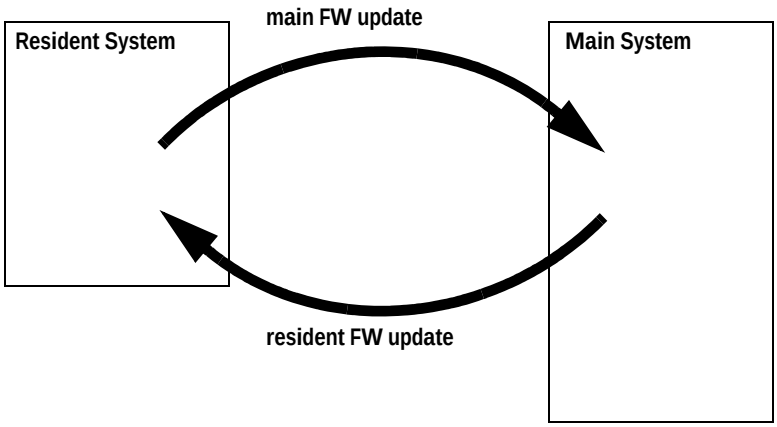

Figure 92 Firmware Update Mechanism

Raw Data Conversion to Fluorescence (LU) Units

LU (light or luminescence units) is used as new unit and will indicate the light intensity (not comparable with the physical unit Lumen!).

These units are calculated from the PMT (photomuliplier tube) in the emission path and a reference diode located behind the flow cell, see figure below.

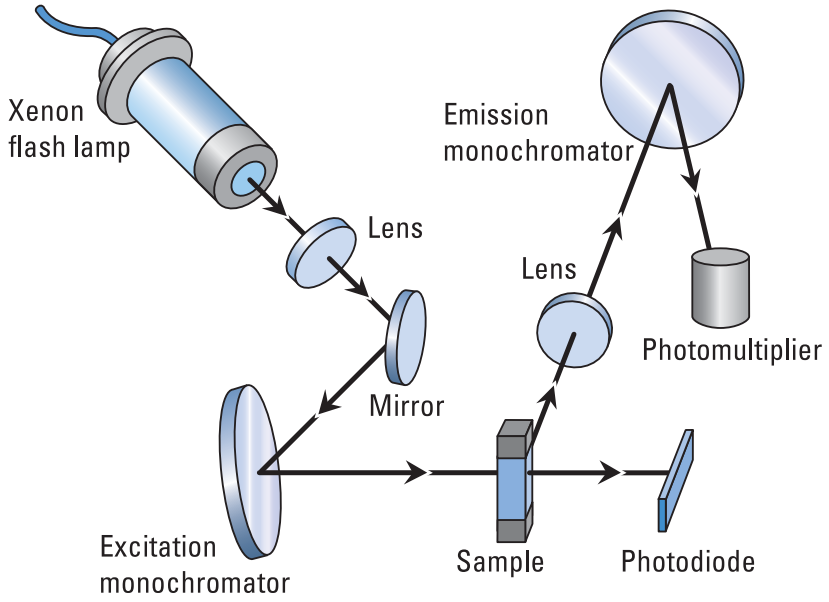

Figure 93 Optical Diagram of the FLD

Data flow for chromatographic output

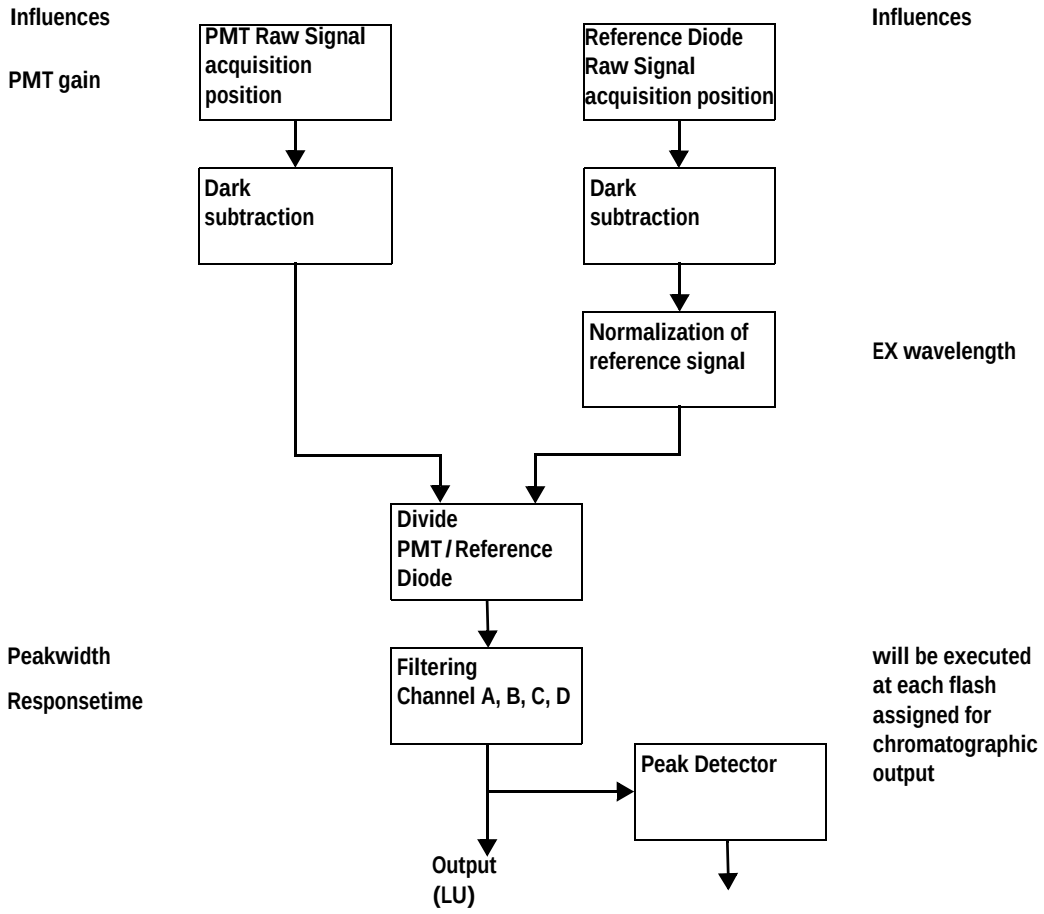

Figure 94 Data flow for chromatographic output

For multi-channel output, any single flash data point will be processed in a separate independent filter.

Data flow for spectral output

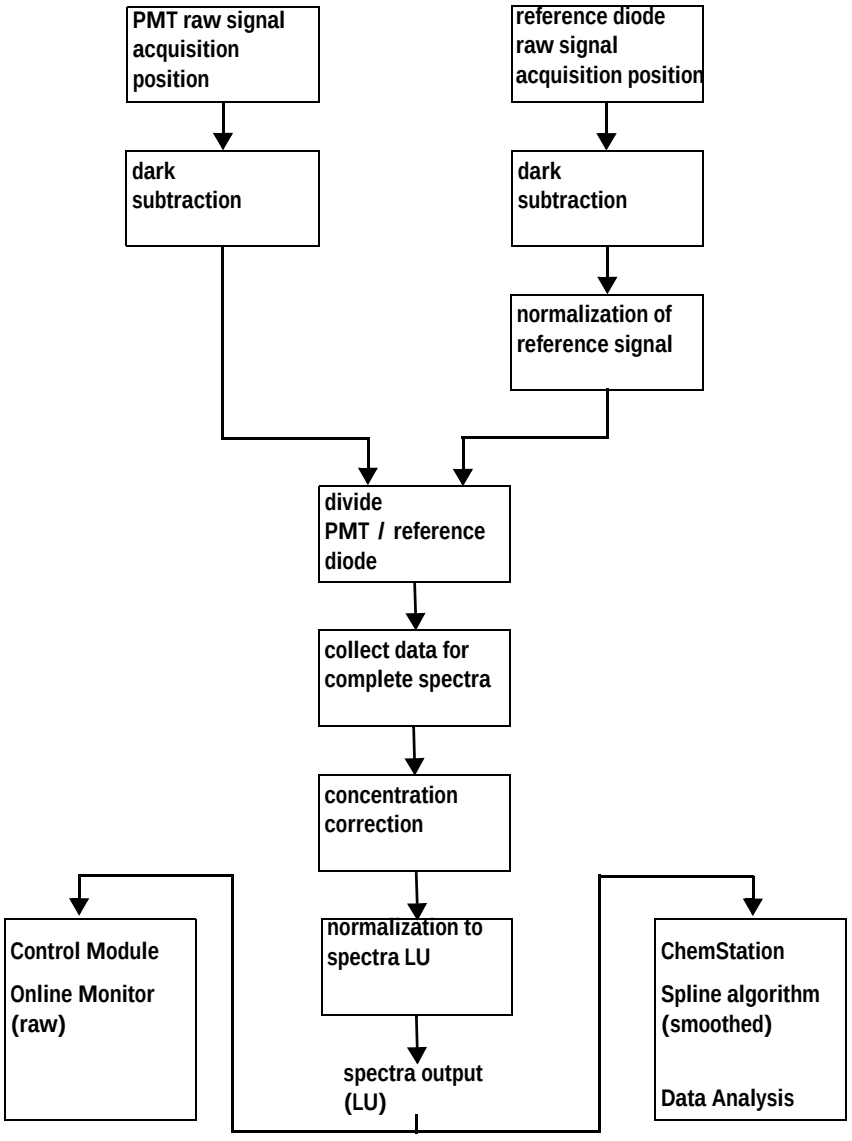

Figure 95 Data flow for spectral output

Raw Signal Acquisition (PMT and Reference Diode)

Each raw data value of the PMT and the reference diode signal is measured synchronized to the flashes of the xenon flash lamp with a 16-bit analog-to-digital converter.

Dark Subtraction

The dark value of the PMT and the reference signal are measured during any power cycle of the detector without flash lamp on. These values are stored and subtracted from any PMT and reference diode value

Normalize Reference Diode Signal

The reference diode, located behind the flow cell, measures the excitation (EX) light within the flow cell and corrects flash lamp fluctuations. Because of a non-linear output of the diode (depending on the EX-wavelength), the measured data are now normalized to get better real light level values within the flow cell.

Divide PMT / Reference Diode

With this calculation the output of the detector will suppress lamp effects like lamp flash fluctuations or lamp lifetime.

Filtering

FIR-filters (finite-impulse-response) are used to filter the given raw data and output it to a the digital data bus and the analog outputs.

Spectra: Collect data to complete spectra

All flashes that are measured to get spectral information are collected to get one spectra.

Concentration Correction

For every measured spectrum, a certain time is required. During this time the concentration within the flow cell may be changed. This can effect the spectra. To avoid this distortion every spectra point is corrected with the quasi-simultaneous measured concentration of the chromatographic channel A. As point of reference the mid-point of each spectra is used.

Concentration correction is not done

- If the cuvette is used, e.g. fluorescence scan.
- If 'all spectra without signal' is used as spectra acquisition mode.

Peak Detector

The peak detector (PD) always uses signal A as the *pilot* signal. The PD is adjusted by means of two setpoints: peakwidth and threshold. Both setpoints are time-programmable. While the PD-threshold is a method parameter of its own, the PD-peakwidth parameter is derived from the general peakwidth (PKWD) parameter. At the start of the run, the value for the PD-peakwidth is set equal to the PKWD setpoint. The peak detector recognizes changes in the slope of this signal (upslope, apex, downslope and baseline) and stores spectra according to the operator's instructions. During the run the PD-peakwidth parameter can be changed by time-programming.

Spectra Acquisition Modes

The spectra acquisition mode allows automatic storage of spectra during a run. The mode can be changed during the run by time-programming.

The modes in detail are:

Table 43 Spectra Acquisition Modes

Mode	Description
none	no spectra is stored
apex	if apex of a peak is detected, one spectra is measured and stored. In addition the next baseline spectra is stored.
all in peak	The peak detectors will start and stop spectrum acquisition at begin and end of a detected peak. In addition the next baseline spectra is stored.
all	periodically all spectra are stored. The period depends on the spectra range and step and the number of selected chromatographical outputs (signals).
all without signals	- for screening all flashes are used for spectra acquisition. - no concentration connection is done. - as chromatographical signal the mean of each spectra is output/stored.

Optional Interface Boards

The Agilent 1200 Series modules have one optional board slot that allows addition of an interface board to the modules.

Table 44 Optional Interface Boards

Description	Part Number
BCD Board	G1351-68701
Fuse 250 mA (four are on the board)	2110-0004
LAN Communication Interface Board	G1369A or G1369-60001

BCD Board

The BCD board provides a BCD output for the bottle number of the Agilent 1200 Series autosampler and four external contacts. The external contact closure contacts are relay contacts. The maximum settings are: 30 V (AC/DC); 250 mA (fused).

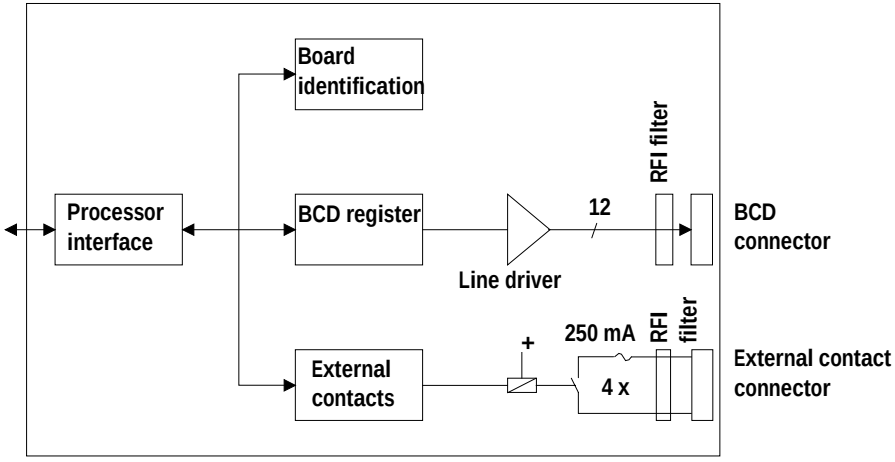

Figure 96 Block Diagram BCD Board

There are general purpose cables available to connect the BCD output, see “BCD Cables” on page 288 and the external outputs, see “External Contact Cable” on page 292 to external devices.

Table 45 Detailed connector layout (1200)

Pin	Signal name	BCD digit
1	BCD 5	20
2	BCD 7	80
3	BCD 6	40
4	BCD 4	10
5	BCD 0	1
6	BCD 3	8
7	BCD 2	4
8	BCD 1	2
9	Digital ground	
10	BCD 11	800
11	BCD 10	400
12	BCD 9	200
13	BCD 8	100
14	not connected	
15	+5V	

LAN Communication Interface Board

NOTE One board is required per Agilent 1200 stack. It is recommended to add the LAN board to the detector with highest data rate.

NOTE The LAN board can only be used together with:

a main board version G13XX-66520 (for G1315A, G1365A, G1314A, G1310A, G1311A, G1312A and G1313A) or newer and on all other modules.

a DOS-ChemStation software revision A.06.01 or above.

The following cards can be used with the Agilent 1200 Series modules.

Table 46 LAN Boards

Type	Vendor	Supported networks
G1369A G1369-60001	Agilent Technologies	Fast Ethernet, Ethernet/802.3, RJ-45 (10/100Base-TX) **recommended for re-ordering**
J4106A (*)	Hewlett Packard	Ethernet/802.3, RJ-45 (10Base-T(
J4105A (*)	Hewlett Packard	Token Ring/802.5, DB9, RJ-45 (10Base-T)
J4100A (*)	Hewlett Packard	Fast Ethernet, Ethernet/802.3, RJ-45 (10/100Base-TX) + BNC (10Base2)

NOTE These cards (*) may be longer orderable. Minimum firmware of the JetDirect cards is A.05.05.

Recommended Cables

Cross-over network cable (shielded, 3 m long), (for point to point connection)	5023-0203
Twisted pair network cable (shielded, 7 m long) (for hub connections)	5023-0202

Interfaces

The Agilent 1200 Series modules provide the following interfaces:

Table 47 Agilent 1200 Series Interfaces

Interface Type	Pumps	Autosampler	DA Detector MW Detector FL Detector	VW Detector RI Detector	Thermostatted Column Compartment	Vacuum Degasser
CAN	Yes	Yes	Yes	Yes	Yes	No
GPIB	Yes	Yes	Yes	Yes	Yes	No
RS-232C	Yes	Yes	Yes	Yes	Yes	No
Remote	Yes	Yes	Yes	Yes	Yes	Yes
Analog	Yes	No	2 ×	1 ×	No	Yes*
Interface board	Yes	Yes	Yes	Yes	No	No

* The vacuum degasser will have a special connector for specific use. For details see description of main board.

- CAN connectors as interface to other Agilent 1200 Series modules,
- GPIB connector as interface to the Agilent ChemStation,
- RS-232C as interface to a computer,
- REMOTE connector as interface to other Agilent products,
- Analog Output connector(s) for signal output, and
- Interface slot for specific interfacing (external contacts, BCD, LAN and so on).

For identification and location of the connectors Figure 24 on page 55.

WARNING **Never use cables other than the ones supplied by Agilent Technologies to ensure proper functionality and compliance with safety or EMC regulations, see "Cable Overview" on page 278.**

Analog Signal Output

The analog signal output can be distributed to a recording device. For details refer to the description of the module's main board.

GPIB Interface

The GPIB connector is used to connect the module with a computer. The address and control switches next to the GPIB connector determine the GPIB address of your module. The switches are preset to a default address and recognized by the operating software from Agilent Technologies.

Table 48 Default Addresses

Autosampler	28	Autosampler	28
Pump	22	RID	29
FLD	23		
VWD	24	Autosampler (HP 1050)	18
Agilent 8453A	25	Pump (HP 1050)	16
DAD / MWD	26	VWD (HP 1050)	10
Column Compartment	27	DAD (HP 1050)	17

CAN Interface

The CAN is an intermodule communication interface. It is a 2-wire serial bus system supporting high speed data communication and real-time requirement.

Remote Interface

The APG remote connector may be used in combination with other analytical instruments from Agilent Technologies if you want to use features such as common shut down, prepare, and so on.

Remote control allows easy connection between single instruments or systems to ensure coordinated analysis with simple coupling requirements.

The subminiature D connector is used. The module provides one remote connector which is inputs/outputs (wired-or technique).

To provide maximum safety within a distributed analysis system, one line is dedicated to SHUT DOWN the system's critical parts in case any module detects a serious problem. To detect whether all participating modules are switched on or properly powered, one line is defined to summarize the POWER ON state of all connected modules. Control of analysis is maintained by signal readiness READY for next analysis, followed by START of run and optional STOP of run triggered on the respective lines. In addition, PREPARE and START REQUEST may be issued. The signal level is defined as:

- standard TTL levels (0 V is logic true, + 5 V is false),
- fan-out is 10,
- input load is 2.2 kOhm against + 5 V, and
- outputs are open collector type, inputs/outputs (wired-or technique).

Table 49 Remote Signal Distribution

Pin	Signal	Description
1	DGND	Digital ground
2	PREPARE	(L) Request to prepare for analysis (for example, calibration, detector lamp on). Receiver is any module performing pre-analysis activities.
3	START	(L) Request to start run / timetable. Receiver is any module performing run-time controlled activities.
4	SHUT DOWN	(L) System has serious problem (for example, leak: stops pump). Receiver is any module capable to reduce safety risk.
5		Not used

Table 49 Remote Signal Distribution (continued)

Pin	Signal	Description
6	POWER ON	(H) All modules connected to system are switched on. Receiver is any module relying on operation of others.
7	READY	(H) System is ready for next analysis. Receiver is any sequence controller.
8	STOP	(L) Request to reach system ready state as soon as possible (for example, stop run, abort or finish and stop injection). Receiver is any module performing run-time controlled activities.
9	START REQUEST	(L) Request to start injection cycle (for example, by start key on any module). Receiver is the autosampler.

RS-232C

The RS-232C connector is used to control the instrument from a computer through RS-232C connection, using the appropriate software. This connector can be activated by the configuration switch module next to the GPIB connector.

The RS-232C is designed as DCE (Data Communication Equipment) with a 9-pin male SUB-D type connector. The pins are defined as follows:

Table 50 RS-232C Connection Table

Pin	Direction	Function
1	In	DCD
2	In	RxD
3	Out	TxD
4	Out	DTR
5		Ground
6	In	DSR
7	Out	RTS
8	In	CTS
9	In	RI

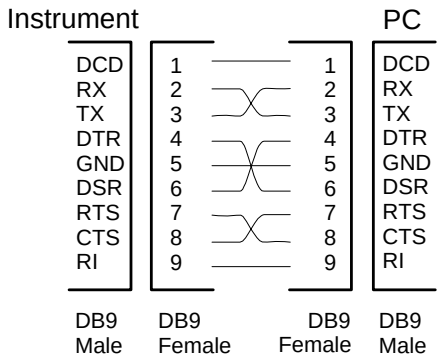

Figure 97 RS-232 Cable

Setting the 8-bit Configuration Switch

The 8-bit configuration switch is located next to the GPIB connector. Switch settings provide configuration parameters for GPIB address, serial communication protocol and instrument specific initialization procedures.

Factory setting is shown for the fluorescence detector

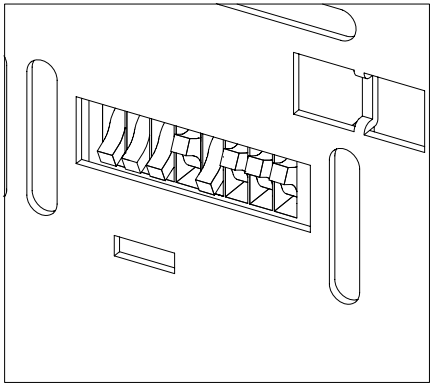

Figure 98 8-bit Configuration Switch

Table 51 8-bit Configuration Switch

Mode Select	1	2	3	4	5	6	7	8
GPIB	0	0		GPIB Address				
RS-232C	0	1	Baud rate			Data Bits	Parity	
Reserved	1	0	Reserved					
TEST/BOOT	1	1	RSVD	SYS		RSVD	RSVD	FC

Switches 1 and 2 define which set of parameters (for example, for GPIB, RS-232C, and so on) will be changed. Once the change has been completed, the instrument must be powered up again in order to store the values in the non-volatile memory.

In the non-volatile memory, the parameters are kept, regardless of whether you turn the instrument off and on again. They will be kept until the same set of parameters is changed and the power is reset. All other previously stored configuration settings will still remain in the non-volatile memory.

In this way, you can store more than one set of parameters using the same 8-bit configuration switch twice, for example, for both GPIB and RS-232C.

GPIB Default Addresses

If you just want to change the GPIB address and need a detailed procedure, refer to the *Installing Your Agilent ChemStation System* handbook.

Default GPIB address is set to the following addresses:

Table 52 Default Addresses for Agilent 1200 Series Modules

Module	Address	Binary Address
Pump	22	0 0 0 1 0 1 1 0
FLD	23	0 0 0 1 0 1 1 1
VWD	24	0 0 0 1 1 0 0 0
Agilent 8453A	25	0 0 0 1 1 1 0 1
DAD / MWD	26	0 0 0 1 1 0 1 0
Column compartment	27	0 0 0 1 1 0 1 1
Autosampler	28	0 0 0 1 1 1 0 0
RID	29	0 0 0 1 1 1 0 1

where 0 means that the switch is down and 1 means that the switch is up.

Communication Settings for RS-232C Communication

The communication protocol used in this instrument supports only hardware handshake (CTS/RTS).

Switches 1 in down and 2 in up position define that the RS-232C parameters will be changed. Once the change has been completed, the instrument must be powered up again in order to store the values in the non-volatile memory.

Table 53 Communication Settings for RS-232C Communication

Mode Select	1	2	3	4	5	6	7	8
RS-232C	0	1	Baud rate			Data Bits	Parity	

Use the following tables for selecting the setting which you want to use for RS-232C communication. The number 0 means that the switch is down and 1 means that the switch is up.

Table 54 Baud Rate Settings

Switches			Baud Rate	Switches			Baud Rate
3	4	5		3	4	5	
0	0	0	9600	1	0	0	9600
0	0	1	1200	1	0	1	14400
0	1	0	2400	1	1	0	19200
0	1	1	4800	1	1	1	38400

Table 55 Data Bit Settings

Switch 6	Data Word Size
0	7 Bit Communication
1	8 Bit Communication

Table 56 Parity Settings

Switches		Parity
7	**8**	
0	0	No Parity
1	0	Odd Parity
1	1	Even Parity

One start bit and one stop bit are always used (not selectable).

Per default, the module will turn into 19200 baud, 8 data bit with no parity.

Forced Cold Start Settings

Switches 1 and 2 do not force storage of this set of parameters in non-volatile memory. Returning switches 1 and 2 to other positions (other than being both up) will allow for normal operation.

CAUTION Forced cold start erases all methods and data stored in the non-volatile memory. Exceptions are diagnose and repair log books which will not be erased.

If you use the following switch settings and power the instrument up again, a forced cold start has been completed.

Table 57 Forced Cold Start Settings

Mode Select	1	2	3	4	5	6	7	8
TEST/BOOT	1	1	0	0	0	0	0	1

To return to normal operation, set switches back to your GPIB or RS 232 configuration settings.

Stay-Resident Settings

Firmware update procedures may require this mode in case of firmware loading errors.

Switches 1 and 2 do not force storage of this set of parameters in non-volatile memory. Returning switches 1 and 2 to other positions (other than being both up) will allow for normal operation.

If you use the following switch settings and power the instrument up again, the instrument firmware stays in the resident part, that is, it is not operable as a detector. It only uses basic functions of the operating system for example, for communication.

Table 58 Stay Resident Settings

Mode Select	1	2	3	4	5	6	7	8
TEST/BOOT	1	1	0	0	1	0	0	0

To return to normal operation, set switches back to your GPIB or RS-232C configuration settings.

The Main Power Supply Assembly

The main power supply comprises a closed assembly (no onsite repair possibility).

The power supply provides all DC voltages used in the module except for the voltages supplied by the lamp power supply to the deuterium and tungsten lamps in the detectors. The line voltage can vary in a range from 100 – 240 volts AC ± 10 % and needs no manual setting.

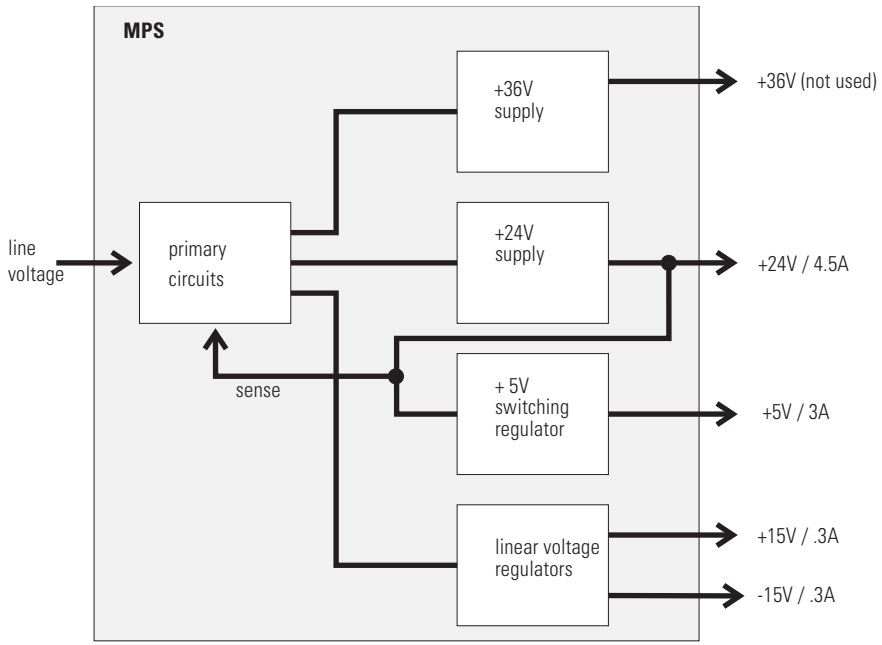

Figure 99 Main Power Supply (MPS) Blockdiagram

To disconnect the instrument from line, unplug the power cord. The power supply still uses some power, even if the power switch on the front panel is turned OFF.

No accessible hardware fuse is needed because the main power supply is safe against any short circuits or overload conditions on the output lines. When overload conditions occur, the power supply turns off all output voltages. Turning the line power off and on again resets the power supply to normal operation if the cause of the overload condition has been removed.

An over-temperature sensor in the main power supply is used to turn off output voltages if the temperature exceeds the acceptable limit (for example, if the cooling fan of the instrument fails). To reset the main power supply to normal operating conditions, turn the instrument off, wait until it is approximately at ambient temperature and turn the instrument on again.

The following table gives the specifications of the main power supply.

Table 59 Main Power Supply Specifications

Maximum power	130 W	Continuous output
Line Input	100 – 240 volts AC ± 10 %, line frequency of 50/60 Hz	Wide ranging
Output 1	+ 24 V / 4.5 A (maximum)	total power consumption of + 24 V and + 36 V must not exceed 107 W.
Output 2	+ 36 V / 2.5 A (maximum)	
Output 3	+ 5 V / 3 A	
Output 4	+ 15 V / 0.3 A	
Output 5	- 15 V / 0.3 A	

A
Appendix

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UV-Radiation (UV-lamps only) 334
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This chapter provides safetey and other general information.

General Safety Information

The following general safety precautions must be observed during all phases of operation, service, and repair of this instrument. Failure to comply with these precautions or with specific warnings elsewhere in this manual violates safety standards of design, manufacture, and intended use of the instrument. Agilent Technologies assumes no liability for the customer's failure to comply with these requirements.

General

This is a Safety Class I instrument (provided with terminal for protective earthing) and has been manufactured and tested according to international safety standards.

This instrument is designed and certified as a general purpose laboratory instrument for research and routine application only. It is not certified for in-vitro or medical applications.

Operation

Before applying power, comply with the installation section. Additionally the following must be observed.

Do not remove instrument covers when operating. Before the instrument is switched on, all protective earth terminals, extension cords, auto-transformers, and devices connected to it must be connected to a protective earth via a ground socket. Any interruption of the protective earth grounding will cause a potential shock hazard that could result in serious personal injury. Whenever it is likely that the protection has been impaired, the instrument must be made inoperative and be secured against any intended operation.

Make sure that only fuses with the required rated current and of the specified type (normal blow, time delay, and so on) are used for replacement. The use of repaired fuses and the short-circuiting of fuseholders must be avoided.

CAUTION The operator of this instrument is advised that if the equipment is used in a manner not specified in this manual, the protection provided by the equipment may be impaired.

Some adjustments described in the manual, are made with power supplied to the instrument, and protective covers removed. Energy available at many points may, if contacted, result in personal injury.

Any adjustment, maintenance, and repair of the opened instrument under voltage should be avoided as much as possible. When inevitable, this should be carried out by a skilled person who is aware of the hazard involved. Do not attempt internal service or adjustment unless another person, capable of rendering first aid and resuscitation, is present. Do not replace components with power cable connected.

Do not operate the instrument in the presence of flammable gases or fumes. Operation of any electrical instrument in such an environment constitutes a definite safety hazard.

Do not install substitute parts or make any unauthorized modification to the instrument.

Capacitors inside the instrument may still be charged, even though the instrument has been disconnected from its source of supply. Dangerous voltages, capable of causing serious personal injury, are present in this instrument. Use extreme caution when handling, testing and adjusting.

Safety Symbols

Table 60 shows safety symbols used on the instrument and in the manuals.

Table 60 Safety Symbols

Symbol	Description
	The apparatus is marked with this symbol when the user should refer to the instruction manual in order to protect the apparatus against damage.
	Indicates dangerous voltages.

Table 60 Safety Symbols (continued)

Symbol	Description
	Indicates a protected ground terminal.
	Eye damage may result from directly viewing the light produced by the deuterium lamp used in this product. Always turn off the deuterium lamp before opening the metal lamp door on the side of the instrument.

WARNING **A warning alerts you to situations that could cause physical injury or damage to the equipment. Do not proceed beyond a warning until you have fully understood and met the indicated conditions.**

CAUTION A caution alerts you to situations that could cause a possible loss of data. Do not proceed beyond a caution until you have fully understood and met the indicated conditions.

Lithium Batteries Information

WARNING **Danger of explosion if battery is incorrectly replaced. Replace only with the same or equivalent type recommended by the equipment manufacturer. Lithium batteries may not be disposed-off into the domestic waste.**

Transportation of discharged Lithium batteries through carriers regulated by IATA / ICAO, ADR, RID, IMDG is not allowed. Discharged Lithium batteries shall be disposed off locally according to national waste disposal regulations for batteries.

WARNING **Lithiumbatteri - Eksplosionsfare ved fejlagtig håndtering. Udskiftning må kun ske med batteri af samme fabrikat og type. Lever det brugte batteri tilbage til leverandøren.**

WARNING **Lithiumbatteri - Eksplosionsfare. Ved udskiftning benyttes kun batteri som anbefalt av apparatfabrikanten. Brukt batteri returneres appararleverandoren.**

NOTE Bij dit apparaat zijn batterijen geleverd. Wanneer deze leeg zijn, moet u ze niet weggooien maar inleveren als KCA.

Radio Interference

Never use cables other than the ones supplied by Agilent Technologies to ensure proper functionality and compliance with safety or EMC regulations.

Test and Measurement

If test and measurement equipment is operated with equipment unscreened cables and/or used for measurements on open set-ups, the user has to assure that under operating conditions the radio interference limits are still met within the premises.

Sound Emission

Manufacturer's Declaration

This statement is provided to comply with the requirements of the German Sound Emission Directive of 18 January 1991.

This product has a sound pressure emission (at the operator position) < 70 dB.

- Sound Pressure Lp < 70 dB (A)
- At Operator Position
- Normal Operation
- According to ISO 7779:1988/EN 27779/1991 (Type Test)

UV-Radiation (UV-lamps only)

Emissions of ultraviolet radiation (200-315 nm) from this product is limited such that radiant exposure incident upon the unprotected skin or eye of operator or service personnel is limited to the following TLVs (Threshold Limit Values) according to the American Conference of Governmental Industrial Hygienists:

Table 61 UV-Radiation Limits

Exposure / day	Effective Irradiance
8 hours	0.1 µW / cm^2
10 minutes	5.0 µW / cm^2

Typically the radiation values are much smaller than these limits:

Table 62 UV-Radiation Typical Values

Position	Effective Irradiance
Lamp installed, 50-cm distance	average 0.016 µW / cm^2
Lamp installed, 50-cm distance	maximum 0.14 µW / cm^2

Solvent Information

Observe the following recommendations on the use of solvents.

Flow Cell

Avoid the use of alkaline solutions (pH > 9.5) which can attack quartz and thus impair the optical properties of the flow cell.

Prevent any crystallization of buffer solutions. This will lead into a blockage/damage of the flow cell.

If the flow cell is transported while temperatures are below 5 degree C, it must be assured that the cell is filled with alcohol.

Aqueous solvents in the flow cell can built up algae. Therefore do not leave aqueous solvents sitting in the flow cell. Add small % of organic solvents (e.g. Acetonitrile or Methanol ~5%).

Solvents

Brown glass ware can avoid growth of algae.

Always filter solvents, small particles can permanently block the capillaries. Avoid the use of the following steel-corrosive solvents:

- Solutions of alkali halides and their respective acids (for example, lithium iodide, potassium chloride, and so on).
- High concentrations of inorganic acids like nitric acid, sulfuric acid especially at higher temperatures (replace, if your chromatography method allows, by phosphoric acid or phosphate buffer which are less corrosive against stainless steel).
- Halogenated solvents or mixtures which form radicals and/or acids, for example:

  $2CHCl_3 + O_2 \rightarrow 2COCl_2 + 2HCl$

  This reaction, in which stainless steel probably acts as a catalyst, occurs quickly with dried chloroform if the drying process removes the stabilizing alcohol.

- Chromatographic grade ethers, which can contain peroxides (for example, THF, dioxane, di-isopropylether) such ethers should be filtered through dry aluminium oxide which adsorbs the peroxides.
- Solutions of organic acids (acetic acid, formic acid, and so on) in organic solvents. For example, a 1-% solution of acetic acid in methanol will attack steel.
- Solutions containing strong complexing agents (for example, EDTA, ethylene diamine tetra-acetic acid).
- Mixtures of carbon tetrachloride with 2-propanol or THF.

Agilent Technologies on Internet

For the latest information on products and services visit our worldwide web site on the Internet at:

http://www.agilent.com

Select “**Products**” - “**Chemical Analysis**”

It will provide also the latest firmware of the Agilent 1200 Series modules for download.

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In This Book

This manual contains technical reference information about the Agilent 1200 Series fluorescence detector.

The manual describes the following:

- introcduction and specifications,
- installation,
- using and optimizing,
- troubleshooting and diagnose,
- maintenance and repair,
- parts identification,
- hardware information,
- safety and related information.

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